日中笹川医学奨学金制度（共同研究コース）研究報告書

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第 40 期　研究者番号(研究者编号)：4016　作成日(书写日期)：2018 年 11 月 19 日

氏　名 (姓名)	吴江	性別 (性别)	男	生年月日 (出生日期)	1978-03-12

研 究 テーマ (研 究 题 目)	Fabrication of novel nanoparticles and its release character
研究期間(来日～帰国まで) (来日起至回国的研究起止时间)	2018 年 08 月 01 日 ～2018 年 12 月 26 日
在日共同研究機関・部署 (在日共同研究单位及部门)	広島大学大学院医歯薬保健学研究科生体材料学
共同研究者氏名・役職 (共同研究者姓名/职务)	加藤 功一 教授　歯科部長
学会参加について (关于在日期间参加学会)	有り(有参加) ☒　　なし(没有参加) ☐ ※参加有の方は下記の欄をご記入下さい。(有参加学会者，请继续填写如下内容)
一般参加 (普通参加)	学会名称：
一般参加 (普通参加)	学会名称：
発表有り (有发表)	学会名称：Japanese Society for Biomaterials 発表テーマ(发表题目)：The enhanced osseointegration of a novel implant antibacterial coating
発表有り (有发表)	学会名称：12th International Symposium on Nanomedicine 発表テーマ(发表题目)：Fabrication of Novel Double-layered Nanoparticles and Evaluation of its Biomedical Characters in vitro
論文発表について (关于在日期间论文发表)	発表有/投稿中(已发表或投稿中)☐　　発表なし(没有发表) ☒ ※既にもしくは発表予定有りの方は下記の欄をご記入下さい。 (已发表或有论文投稿中的，请填写如下内容，并另外单独附上全部论文内容的复印件)
	テーマ(题目)：
	著者名(作者名)：
	雑誌名(期刊名)：
	発行年(发表年度)： 巻　号(刊卷)： ページ(页数)： インパクトファクター(影响因子)：

日本滞在中の具体的な共同研究内容についての報告
（关于在日期间就研究题目具体开展的共同研究内容的详述）

【研究目的】（研究目的）

To design and fabricate the novel double-layered nanoparticle with sustained release and stem-cell recruitment characters.

【研究経過】（研究经过）

After I arrived at Hiroshima University, Prof. Kato, Hirata and I have discussed about the process. After careful studied the literatures, the protocol was made and the fabrication process was tried. With ultrasonication, 1 μg of SDF-1 was dissolved in 10 mL of polylactic acid nanosphere solution, which was the outer oil phase; 20 mg of chitosan was dissolved in 10 mL of 2% acetic acid solution, which was the outer aqueous phase. The outer layer of the oil phase was added dropwise to the outer aqueous phase at 25 ° C, and 1 mL of a 1% aqueous solution of sodium tripolyphosphate was added as an outer layer crosslinking agent, followed by emulsification. The obtained oil-water emulsion was magnetically stirred at 500 rpm for 12 hours to remove acetic acid.

Besides, I also attended the lab seminar every Thursday and made discussion with other researchers in the lab. And also made presentation of my current research in China.

【成果】（成果）

The design and fabrication process was made and optimized parameters were determined. For inner layer, the PLA concentration in the organic phase was 7.5%, the oil-water ratio was 1:20, the rhBMP-2 dosage was 1.5 μg, and the emulsification time was 200 s. The double layered nanoparticles were made with optimized protocol. The mass of chitosan was 20 mg, the ratio of internal and external liquid was 2:3, the dosage of SDF-1 was 1.0 μg, and the emulsification time was 300 s.

【今後の論文発表予定】（今后论文发表的计划）

After I come back to China, I will continue to cooperate with Prof. Kato on the research and we hope to submit the international paper next year.

【今後の課題】（今后的课题）

In the future, we would still focus on the environment-sensitive nanoparticle and its cell recruitment characters in vivo.

本共同研究の①研究目的の達成度、②将来性、③帰国後の展開予定について述べて下さい。
（请就本次合作研究题目的①研究目的的达标情况、②未来的发展可能性、③您回国后今后的合作研究的规划打算作一论述）

【達成度】（达标情况）

For the research carried out in Japan and China, we have made 40% achievements of the project.

【将来性】（未来的可能性）

In the future, the rest of research including cell recruitment characters, sustained release character in vivo will be done in the next 1 year. And we also hope to apply for the joint project in the future.

【帰国後共同研究の展開予定】（回国后的合作规划）

In the future, we will continue to cooperate on the project on nanoparticles innovation and 2019, The 7th Japan-China Symposium on Nanomedicine will be held in Xi' an. Prof. Kato and Dr. Hirata will join the meeting and give important presentation. I will also join the meeting and invite them to my hospital.

研究者自署：吴江 (印)吴江

日中笹川医学奨学金制度（共同研究コース）
日中共同研究に関わる報告書

Supported by 日本財団 THE NIPPON FOUNDATION

作成日：2019年1月7日

氏 名（漢字）	加藤　功一	氏名（ローマ字）	Koichi Kato
所属機関・部署・役職	広島大学・大学院医歯薬保健学研究科・教授		
研究テーマ	Fabrication of novel nanoparticles and its release character		
中国側共同研究者 氏名と研究者番号	吴江　4016	中国側共同研究者 所属機関	第四军医大学口腔医院

本共同研究の①研究目的の達成度、②将来性、③今後共同研究の展望や予定について述べて下さい。

【達成度】

当初の計画に対して、達成度は40％であった。

【将来性】

本共同研究を継続し、これまでに設計したナノマテリアルがもつ細胞誘因効果を調べるための細胞培養系を用いた評価、及び、動物実験による生体活性物質の徐放効果及び骨再生誘導能の評価について一年以内に実施し成果を得る。また、本共同研究プロジェクトの継続的発展のため、外部資金獲得を目指す。

【今後の展望】

今後も、継続して共同研究を遂行する。2019年5月には、西安で開催される7th Japan-China Symposium on Nanomedicineにおいて、共同研究の成果を発表する予定である。

日本側共同研究者自署：加藤功一 印

日中笹川医学奨学金制度（共同研究コース）研究報告書

＊英語または日本語で作成（请用日文或英文书写）

Supported by 日本財団 THE NIPPON FOUNDATION

第　　期　　研究者番号（研究者编号）：K4017　　作成日（书写日期）：2018.11.24

氏　名（姓名）	CHENG WEI PING	性別（性别）	M	生年月日（出生日期）	1958. 10. 13

研 究 テーマ（研 究 题 目）	A Comparative Analysis of cognitive impairment in elderly in China and Japan
研究期間（来日～帰国まで）（来日起至回国的研究起止时间）	2018.07. 25 ～ 2018. 12. 18
在日共同研究機関・部署（在日共同研究单位及部门）	Prefectural University of Hiroshima, Faculty of Health and Welfare
共同研究者氏名・役職（共同研究者姓名/职务）	Hidetoshi Harada (Professor) Atsushi Hosokawa (Senior Lecturer)

学会参加について（关于在日期间参加学会）

有り（有参加）　　なし（没有参加）

※参加有の方は下記の欄をご記入下さい。（有参加学会者，请继续填写如下内容）

一般参加（普通参加）	学会名称：
一般参加（普通参加）	学会名称：
発表有り（有发表）	学会名称：The Third Industry-Academia Collaboration Research Workshop 発表テーマ（发表题目）：1. Characteristics of Constitution in Chinese Medicine Questionnaire of the aged in Harbin, China 2. A study on the correlation between Constitution in Chinese Medicine Questionnaire (CCMQ) of the aged in Harbin, China and depression state 3. A study on the correlation between Constitution in Chinese Medicine Questionnaire (CCMQ) of the aged in Harbin, China and cognitive function 4. A study on the correlation between Constitution in Chinese Medicine Questionnaire (CCMQ) of the aged in Harbin, China and activity of daily life (ADL)
発表有り（有发表）	学会名称： 発表テーマ（发表题目）：

論文発表について（关于在日期间论文发表）

発表有/投稿中（已发表或投稿中）　　発表なし（没有发表）

※既にもしくは発表予定有りの方は下記の欄をご記入下さい。
（已发表或有论文投稿中的，请填写如下内容，并另外单独附上全部论文内容的复印件）

テーマ（题目）：

著者名（作者名）：

雑誌名（期刊名）：

発行年（发表年度）：

巻　号（刊卷）：

ページ（页数）：

インパクトファクター（影响因子）：

日本滞在中の具体的な共同研究内容についての報告
（关于在日期间就研究题目具体开展的共同研究内容的详述）

【研究目的】（研究目的）

Constitution in Chinese Medicine Questionnaire (CCMQ), MoCA, MMSE, AD8, AIS and ADL were used to study constitution, cognitive function and the ability of daily life of elderly people in China and Japan, so as to provide better methods for preventing cognitive impairment of elderly people in China and Japan.

【研究経過】（研究经过）

The research is mainly studied from three aspects.

1.A comparative analysis of cognitive impairment between China and Japan.

2.A comparative analysis on the prevention and treatment of cognitive impairment between China and Japan.

3.Compare and analyze characteristics of traditional Chinese medicine (TCM) constitution of patients of cognitive impairment between China and Japan.

【成果】（成果）

1. Characteristics of nine types of TCM constitution in elderly in Harbin, China
2. Relationship among TCM constitution, cognitive function, the ability of daily life, depression, sleep and mental state in elderly people in Harbin, China

【今後の論文発表予定】（今后论文发表的计划）

1. Prepare to publish 2 or 3 papers in Chinese domestic academic journals (2019-2020)
2. Prepare to publish 2 or 3 papers in Japanese domestic journals (2019-2020)
3. Plan to publish 1 or 2 papers in English journal (2019-2020)

【今後の課題】（今后的课题）

1. A Comparative Analysis of cognitive impairment in elderly between China and Japan

Continue to complete the cooperative projects, such as increasing the data of the research objects, expanding the sample size, and analyzing the research content from multiple levels and angles to obtain mutual relations

2.A comparative study on the prevention of cognitive impairment by adjusting the nine constitutions of elderly people in China and Japan.

本共同研究の①研究目的の達成度、②将来性、③帰国後の展開予定について述べて下さい。
（请就本次合作研究题目的①研究目的的达标情况、②未来的发展可能性、③您回国后今后的合作研究的规划打算作一论述）

【達成度】（达标情况）
According to the research plan, we have completed the investigation of nine types of TCM constitution (500 cases), and made a preliminary analysis on the correlation among MoCA, MMSE, ADL and AD8. We have written four papers, which are ready to be published at the third industry-academia collaboration research workshop in Hiroshima, Japan.

【将来性】（未来的可能性）
1.Pleasant cooperation with Japanese co-researchers, which is the foundation of future joint research.
2.To explore the constitution of elderly cognitive impairment's patients through the studies on the constitution of elderly people in China and Japan, so as to lay a theoretical foundation of methods for the prevention and treatment of elderly people with cognitive impairment in future.

【帰国後共同研究の展開予定】（回国后的合作规划）
1. Further sorted out, statistically analyzed and analyzed the data of Chinese and Japanese elderly constitution, MoCA, MMSE, AD8 and ADL obtained in the past six months.
2. Write papers based on the results of the joint research and publish them in different academic journals
3. On the basis of the previous stage of research, further cooperation will be carried out in the prevention and treatment of cognitive impairment in elderly people in China and Japan.
4. Invite the Japanese partner to attend the academic conference held by China and make a keynote speech about the joint research content.

研究者自署： ㊞

日中笹川医学奨学金制度（共同研究コース）
日中共同研究に関わる報告書

Supported by 日本財団 THE NIPPON FOUNDATION

作成日 2018 年 12 月 12 日

氏 名（漢字）	原田俊英・細川淳嗣	氏名（ローマ字）	Toshihide HARADA/Atsushi HOSOKAWA
所属機関・部署・役職	県立広島大学　保健福祉学部　理学療法学科　教授 県立広島大学　保健福祉学部　コミュニケーション障害学科　講師		
研究テーマ	中国と日本における老年期認知症の比較研究		
中国側共同研究者 氏名と研究者番号	程　為平　K4017	中国側共同研究者 所属機関	黒龍江中医薬大学 附属第一医院

本共同研究の①研究目的の達成度、②将来性、③今後共同研究の展望や予定について述べて下さい。

【達成度】

二国間の比較をするため、双方の国で標準化されている認知症とその周辺症状についてのスケールを選択した。使用される検査スケールを以下の 7 種に決定した。①Mini-Mental State Examination(MMSE; 認知症スクリーニングスケール)、②The Montreal Cognitive Assessment (MoCA；認知機能検査)、③Dementia Screening Interview(AD8；認知症で発現する行動の障害のチェックリスト)、④Basic Activity of Daily Living (BADL；基本的日常生活動作のスケール)、⑤Instrumental Activity of Daily Living (IADL；日常の応用動作のスケール)、⑥ Athens Insomnia Scale (AIS；睡眠障害のスクリーニングスケール)、⑦Hamilton Depression Scale (HDS；抑うつ状態のスケール)。これらはいずれも日中双方の文化に合わせて訳されておりかつ、国際的にも広く使われているものである。今後、日中間の比較だけでなく、その他の国との比較においても同じ方法での研究が可能になる。

また、本研究の最終到達点を根拠に基づいた中医学による認知症予防・治療に置いている。しかし、現段階では、中医学における代表的な診断法の一つであり漢方の処方や鍼の打ち方の根拠となる「九種体質分類」と認知症との関係性は、学術的に明らかになっていない。そのため、本共同研究では、認知症の症状やその周辺症状や関連障害と九種体質分類との関係を大規模なデータから明らかにすることを目指した。そのため、上記 7 種の検査スケールの利用に加えて、中医学体質アンケート(the Constitution in Chinese Medicine Questionnaire; CCMQ)を利用し、これによる体質と検査スケールとの関係性の検討を行うこととした。なお、CCMQ の原版は中国語で作成されているが、すでに日本語に翻訳され、原版との検査妥当性について先行研究で確認されているため、日中比較が可能である。

上記の諸データの収集を中国側で本共同研究終了までに先行して実施し、現在約 600 名分のデータの収集が終わり、CCMQ と一部の検査スケールとの間で、予備的な分析を行った。その結果、記憶など認知症における中核的な認知機能障害を持った人（MoCA のスコアが低値）においては陰虚質、湿熱質が認知機能正常の人に比べ統計的に有意に多く見られた。今後、詳細な検討が必要であるが、体質と認知症の症状やそれに関連した諸症状や障害との関連性が明らかになる可能性が高い。

これらの初期的な解析の結果については、2018 年 12 月 11 日に県立広島大学にて開催された、第 3 回国際産学連携交流会の中で口頭発表 1 題、ポスター発表 5 題としていずれも英語で発表された。発表には、研究分担者である、黒龍江中医薬大学の程教授の指導学生である大学院生 6 名も来日し、発表するとともに、参加者との議論を行った。

【将来性】

本共同研究により、根拠に基づいた中医学による認知症治療のための第一段階への見通しがついた。今後、小規模な体質に応じた予防・治療の効果の研究、比較対照群を設けての予防・治療研究への展開へと繋げていく。

【今後の展望】

今後の本共同研究の継続や業績の発表について、以下のように予定している。

1. 本共同研究期間に収集されたデータの詳細な分析と中医学専門雑誌（英文）への投稿
2. 上記と同様の方法による日本側でのデータ収集と分析および、中国側データとの比較分析とそれに関する論文投稿
3. 本学と黒龍江中医薬大学との認知症治療を中心とした大学院生など学生も含んだ継続的な双方的な交流

日本側共同研究者自署：原田俊英 印

日中笹川医学奨学金制度(共同研究コース)研究報告書

Supported by 日本財団 THE NIPPON FOUNDATION

＊英語または日本語で作成(请用日文或英文书写)

第 40 期　研究者番号(研究者编号)：K4018　作成日(书写日期)：2018 年 10 月 17 日

氏　名 (姓名)	Liqun Yang	性別 (性别)	Male	生年月日 (出生日期)	1982.07.06

研 究 テーマ (研 究 题 目)	Biodegradable oral delivery system based on butyrate-functionalized PTMC particles for inflammatory bowel disease therapy
研究期間(来日～帰国まで) (来日起至回国的研究起止时间)	2018 年 5 月 21 日 ～ 2018 年 11 月 27 日
在日共同研究機関・部署 (在日共同研究单位及部门)	Department of Applied Chemistry, Faculty of Engineering, Kyushu University
共同研究者氏名・役職 (共同研究者姓名/职务)	Yoshiki Katayama Dean and Professor, Graduate School of Systems Life Sciences, Kyushu University Director of Center for Advanced Medical Innovation, Kyushu University
学会参加について (关于在日期间参加学会)	有り(有参加) ☒　　なし(没有参加) ☐ ※参加有の方は下記の欄をご記入下さい。(有参加学会者，请继续填写如下内容)
一般参加 (普通参加)	学会名称：The 34th Annual Meeting of the Japan Society of Drug Delivery System
一般参加 (普通参加)	学会名称：67th Symposium on Macromolecules
一般参加 (普通参加)	学会名称：5th TERMIS World Congress
発表有り (有发表)	学会名称：The 40th Annual Meeting of the Japanese Society for Biomaterials 発表テーマ(发表题目)： Biodegradable cross-linked networks based on poly(trimethylene carbonate)
論文発表について (关于在日期间论文发表)	発表有/投稿中(已发表或投稿中)☐　　発表なし(没有发表)☒ ※既にもしくは発表予定有りの方は下記の欄をご記入下さい。 (已发表或有论文投稿中的，请填写如下内容，并另外单独附上全部论文内容的复印件)
	テーマ(题目)：
	著者名(作者名)：
	雑誌名(期刊名)：
	発行年(发表年度)： 巻　号(刊卷)： ページ(页数)： インパクトファクター(影响因子)：

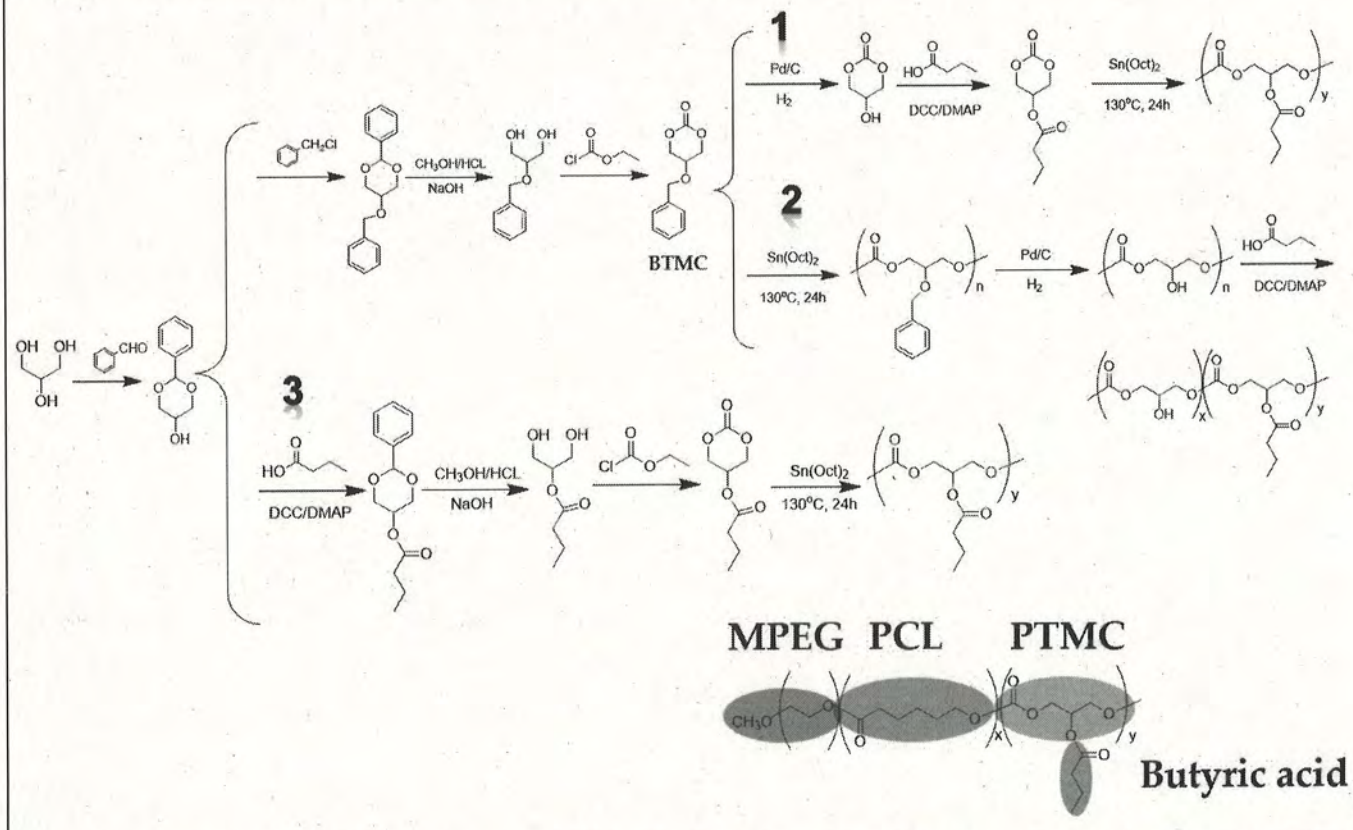
日本滞在中の具体的な共同研究内容についての報告
（关于在日期间就研究题目具体开展的共同研究内容的详述）

【研究目的】(研究目的)

The joint research purpose is to design and construct an oral drug delivery system based on butyric acid-functionalized PTMC nanoparticles for the treatment of inflammatory bowel disease (IBD). IBD has become a global disease, and its incidence rate is still increasing in the world. The recent researches showed that butyrate is useful to the treatment of IBD, but clinical experience shows that the rapid metabolism of butyrate limits its clinical potential, and high does is needed for treatment of IBD, while oral application of butyrate is difficult. How to deliver the butyrate to treat the IBD effectively has become an important problem that needs to be solved urgently. To overcome this problem, we designed a biodegradable oral delivery system based on butyrate-functionalized PTMC particles for therapy of IBD.

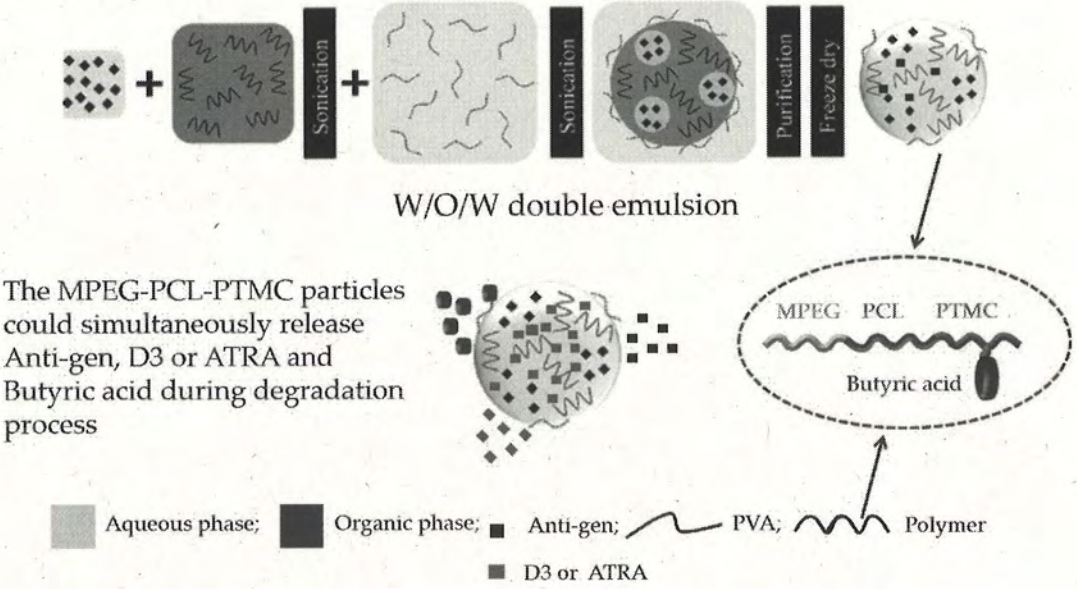
【研究経過】(研究经过)

To obtain this system, we synthesized the biodegradable butyrate-functionalized PTMC as particles carrier, using glycerol as the raw materials. The synthesis rout is as following:



After the protection of hydroxyl groups and the hydrolysis reaction, the obtained product will react with ethyl chloroformate to get the 5-benzyloxyl trimethylene carbonate BTMC. Then the targeted polymer will be got in the follow way. Alternatively, the corresponding polymer can be obtained firstly by ring-opening polymerization of BTMC. After the hydrogenolysis, the product further reacts with butyric acid via esterification, target polymer can also be obtained. In addition, according to the reference, glycerol can be reacted with butyric acid to get a crude product Then the crude is reacted with ethyl chloroformate to get butyrate-functionalized TMC, and the polymers will be obtained via the

ring-opening polymerization. Mpeg and polycaprolactone PCL can be introduced to the structures of butyrate-functionalized PTMC to tailed its degradation rate and the self-assembly behavior. After that, nanoparticles will be prepared by W/O/W double emulsion. The particles could simultaneously release Anti-gen, D3 or ATRA and Butyric acid during degradation process.



【成果】(成果)

During the past 5 months, our effort was devoted to the synthesis and characterization of the intermediated compounds and monomer, and the intermediated compounds have been obtained. Monomer is being synthesized.

【今後の論文発表予定】(今后论文发表的计划)

2~3 SCI papers will be published in the professional journals in next 2 years, and the impact factor of one paper should be higher than 5:

1. Synthesis, characterization and properties of the novel aliphatic polycarbonates based on butyrate functionalized poly(trimethylene carbonate) (European Polymer Journal?)
2. Preparation and characterization of the butyrate functionalized poly(trimethylene carbonate) nanoparticles for the controlled release of vitamin D3 (Polymer?)
3. Therapeutic effect of vitamin D3-containing nanoparticles based on butyrate functionalized poly(trimethylene carbonate) on inflammatory bowel disease (Journal of controlled Release?)

【今後の課題】(今后的课题)

1. Further study on the preparation, properties and the therapeutic effect of vitamin D3-containing nanoparticles based on butyrate functionalized poly(trimethylene carbonate) on inflammatory bowel disease.
2. Stimuli-responsive nanoparticles based on the biodegradable polycarbonate liquid crystals for drug delivery system.

本共同研究の①研究目的の達成度、②将来性、③帰国後の展開予定について述べて下さい。
（请就本次合作研究题目的①研究目的的达标情况、②未来的发展可能性、③您回国后今后的合作研究的规划打算作一论述）

【達成度】(达标情况)

The whole joint research can be divided into 3 parts:

1. Synthesis and properties of the intermediated compounds, monomer and polymers as the drug delivery carriers;
2. The preparation and in vitro release behavior of the vitamin D3 containing nanoparticles;
3. Therapeutic effect of vitamin D3-containing nanoparticles on inflammatory bowel disease;

To our best knowledge, there are no reports about some of the intermediated compounds or the targeted monomer so far. Therefore, the whole research, especially for the preparation of monomer and polymer carrier materials has a high innovation. However, the synthesis and preparation of monomers are relatively difficult, it takes a long time to obtain the final product with correct structure and high purity. During the past 5 months, our effort was devoted to the synthesis and characterization of the intermediated compounds and monomer, and the intermediated compounds have been obtained, and the monomer and polymer carriers are being synthesized in prof. Katayama's lab. About 25% of the whole research purpose has been achieved. The further research will be continued in the next 2 years.

【将来性】(未来的可能性)

The recent researches showed that Butyrate is useful to the treatment of IBD. The evidences showed that: Butyrate may ameliorate IBD by regulating Foxp3 and Butyrate enemas seem to be clinically beneficial for patients with colitis. Butyrate is secreted by bacteria and can be found at high concentration in colon, but patients with IBD have reduced numbers of bacteria that produce butyrate. Hence, Butyrate Supplementary is vital to the IBD patients. However, clinical experience shows that the rapid metabolism of butyrate limits its clinical potential, and high does is needed for treatment of IBD, while oral application of butyrate is difficult. How to deliver the butyrate to treat the IBD effectively has become an important problem that needs to be solved urgently.

To overcome this problem, we designed a biodegradable oral delivery system based on butyrate-functionalized PTMC particles for therapy of IBD. This system has several advantages as following, such as Release of the butyric acid, Containing antigen and D3 or ATRA. Furthermore, it can be degraded in vivo. So, the system may be a promising strategy for therapy of IBD.

【帰国後共同研究の展開予定】(回国后的合作规划)

My visit to Professor Katayama's Lab is just the successful beginning of our cooperation. The further cooperation between my group and Professor Katayama's Lab, Kyushu University and Liaoning research institute of family planning/China Medical University will both be further promoted, which is also important for the academic exchanges between Japan and China, especially in the medical fields. In a word, we will continue to work hard and strive to be better. Hence, the further cooperation plan is as following:

1. The further research about the preparation and effect evaluation will be performed after back to China
2. Prof. Katayama will be invited as Guest Professor in China Medical University
3. Joint training of PhD students (if possible)
4. Application for the national cooperative research program (if possible)

研究者自署：杨子群 ㊞

日中笹川医学奨学金制度（共同研究コース）
日中共同研究に関わる報告書

Supported by 日本財団 THE NIPPON FOUNDATION

作成日：2018 年 11 月 29 日

氏 名（漢字）	片山佳樹	氏名（ローマ字）	KATAYAMA YOSHIKI
所属機関・部署・役職	九州大学大学院・工学研究院・教授		
研究テーマ	Biodegradable oral delivery system based on butyrate-functionalized PTMC particles for inflammatory bowel disease therapy		
中国側共同研究者 氏名と研究者番号	楊立群・K4018	中国側共同研究者 所属機関	Liaoning Research Institute of Family Planning, China Medical University

本共同研究の①研究目的の達成度、②将来性、③今後共同研究の展望や予定について述べて下さい。

【達成度】

本共同研究では、我々の展開している炎症性大腸炎などの難治性炎症性疾患に対して、その免疫系の制御を介して、慢性炎症を根治できるナノメディシンの創成において、中国側共同研究者の有する高分子合成技術を活かして、経口投与において酪酸およびビタミン D3 を腸内に効率よく送達できる独自のナノキャリヤーを開発することを目的としていた。まず、ナノキャリヤーに適した分子設計を行い、その合成を実施した。当初考えていたより、その合成に困難が伴い、何度も異なるアプローチでトライを繰り返し、これに予想外に時間を要した。その結果、高分子キャリヤーのモノマー及びポリマーの合成に成功することができた。

今回合成に成功した高分子キャリヤーは、酪酸を分子内に組み込み、ビタミン D3 を疎水コアに内包でき、両者を腸内まで安定に保護しつつ、疾患部位で徐放できる可能性を有する世界でも初めての材料であり、非常に価値の高いものであると評価出来る。当初予定していた動物モデルでの評価までには到らなかったが、キャリヤー合成できたことで、この後の評価や、それを受けての分子の改善や、治療デバイスとしての開発は、問題無く行えると期待できる。達成度としては、評価まで到らなかったと言うことで 50%程度であるが、困難な合成を克服して高度な機能を有する材料の合成に成功したことは高く評価出来ると考える。

【将来性】

今回合成に成功したキャリヤーは直ちにその性能を評価出来、世界でも例のない炎症性腸疾患を免疫制御により根治できる可能性を秘めたナノキャリヤー実現の可能性を大いに期待できるものである。現在、炎症性腸疾患は、ステロイドなどの強力な抗炎症剤や炎症性サイトカインに対する抗体医薬が用いられるが、持続投与が必要で、効果が限定的であるとともに重篤な副作用を伴うことが多いため、問題が多い。しかし、これまで根治の可能性のある治療デバイスは存在しなかった。今回合成したキャリヤーを用いたナノメディシンは、この問題を解決できる全く新しい治療法を生み出せる可能性を有しており、非常に大きな将来性があると言える。

【今後の展望】

今回、目的とする高分子型ナノキャリヤーの合成に成功したので、 今後は、共同研究としてその性能評価を実施する。高分子キャリヤーを中国で合成して貰い、これを用いて当方で、モデルマウスを用いた治療効果の検証を行う。酪酸とビタミン D3 の徐放能の評価から、分子内に導入する酪酸のモル分率を変化させたり、ナノ粒子の安定性、腸内リパーゼによる薬物放出能の評価などを系統的に行い、相互に連絡をとりつつ、分子をさらに最適化して、有効な治療デバイスの開発につなげていく。また、今後も、相互に滞在しながら、中国側にも評価システムや評価技術を導入するとともに、さらに免疫制御能を有する他の薬剤の内包なども検討していく。この様にして、今回の高分子合成の成功により、これまで困難であった腸管免疫の工学的な制御を可能にし、難治性の炎症性腸疾患の根治を目指す新しい医療技術の開発を進めていく。

日本側共同研究者自署：片山佳樹 ㊞

日中笹川医学奨学金制度(共同研究コース)研究報告書

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　研究者番号(研究者编号)：K4019　作成日(书写日期)：30 年 7 月 17 日

氏　名 (姓名)	胡　英華	性別 (性别)	女	生年月日 (出生日期)	昭和 46 年 7 月 24 日

研 究 テーマ (研 究 题 目)	An accurate measurement of oxidative DNA damage in inflammatory model rat
研究期間(来日～帰国まで) (来日起至回国的研究起止时间)	30 年 5 月 11 日 ～ 30 年 8 月 8 日
在日共同研究機関・部署 (在日共同研究单位及部门)	産業医科大学　産業生態科学研究所　職業性腫瘍学
共同研究者氏名・役職 (共同研究者姓名/职务)	河井　一明　教授
学会参加について (关于在日期间参加学会)	有り(有参加) ×　　なし(没有参加) □ ※参加有の方は下記の欄をご記入下さい。(有参加学会者，请继续填写如下内容)

一般参加 (普通参加)	学会名称： 第 91 回日本産業衛生学会
一般参加 (普通参加)	学会名称：
発表有り (有发表)	学会名称： 発表テーマ(发表题目)：
発表有り (有发表)	学会名称： 発表テーマ(发表题目)：

論文発表について (关于在日期间论文发表)	発表有/投稿中(已发表或投稿中) □　　発表なし(没有发表) × ※既にもしくは発表予定有りの方は下記の欄をご記入下さい。 (已发表或有论文投稿中的，请填写如下内容，并另外单独附上全部论文内容的复印件)
	テーマ(题目)：
	著者名(作者名)：
	雑誌名(期刊名)：
	発行年(发表年度)： 巻　号(刊卷)： ページ(页数)： インパクトファクター(影响因子)：

日本滞在中の具体的な共同研究内容についての報告
（关于在日期间就研究题目具体开展的共同研究内容的详述）

【研究目的】（研究目的）

Oxidative stress is cause of lifestyle-related diseases such as cancer, cranial nerve diseases, cardiac diseases and arteriosclerosis. Inflammation is the most frequent initial symptom of these diseases and closely related to oxidative stress. An accurate measurement and an appropriate assessment of oxidative stress in a body will be very useful for an understanding of pathogenic mechanism and prevention of diseases. In this study, we have investigated an accurate measurement of the oxidative DNA damage in inflammatory model rat (Long-Evans Cinnamon (LEC)).

【研究経過】（研究经过）

By means of high-performance liquid chromatography with electrochemical detection (HPLC-ECD), the oxidative DNA damage was measured in terms of the amount of 8-hydroxydeoxyguanosine (8-OHdG) in nuclear DNA extracted from a 15 weeks' male LEC rat's liver, spleen, kidneys, brain, lung, heart and testis. An age-matched healthy Long-Evans Agouti (LEA) rat was used as control.

【成果】（成果）

8-OHdG levels were significantly higher in LEC liver and kidneys as compared to control respectively. But no significant differences were observed in LEC lung, spleen, brain, heart and testis. These results suggested that oxidative DNA damage were significantly higher in kidney in addition to previously recognized main copper accumulating organ - liver. Oxidative stress DNA damage plays a role on the pathophysiology of Wilson's disease (WD) or even other metal-induced diseases, which should be more broadly studied.

【今後の論文発表予定】（今后论文发表的计划）

1. Cardiac alterations in Wilson's disease——Journals review.

【今後の課題】（今后的课题）

1. Oxidative DNA damage induced by inorganic metal mercury (lead)
2. Oxidative Stress and myocardiosis induced by organophosphorus poisoning

本共同研究の①研究目的の達成度、②将来性、③帰国後の展開予定について述べて下さい。
（请就本次合作研究题目的①研究目的的达标情况、②未来的发展可能性、③您回国后今后的合作研究的规划打算作一论述）

【達成度】（达标情况）

Many carcinogens cause cell mutation by inducing oxidative DNA damage. Among them, the DNA damage induced by reactive oxygen species is the cause of many lifestyle-related diseases. The aim of the present study was to investigate an accurate measurement of 8-hydroxydeoxyguanosine (8-OHdG) as an oxidative stress marker. Based on the fact that inflammation correlate with many diseases, oxidative DNA damage of various organs were measured in inflammatory model rat. All scheduled organs, including no reported tissues, has been analysed. Our measurement values were much lower compared to previous report. We think this may partly due to the artifact reduction in the operating process. The objective of this study has almost been achieved.

【将来性】（未来的可能性）

Up to now, there have been many reports of 8-OHdG. But the measured values were different according to the different measurement methods, this was a big problem. A high-accuracy measurement of 8-OHdG had been studied more than 20 years in our laboratory. As I have learned and mastered this method, I would be able to apply this skill and knowledge for an understanding of pathogenic mechanism and prevention of diseases.

【帰国後共同研究の展開予定】（回国后的合作规划）

As to me it is really a huge harvest of mastering the accurate measurement and appropriate assessment of oxidative stress marker in a body. Our hospital will be benefited from it too. Being an occupational diseases hospital, occupational diseases in China and occupational related diseases are our work task. Not only the understanding of pathophysiology mechanism and proper treatment, but also effective prevention strategy. Based on this common research project, we can apply the obtained knowledge of oxidative stress damage in the treatment, prevention, research of occupational diseases. For oxidative stress is closely associated with so many diseases not only cancers and lifestyle-related diseases but also many occupational diseases. Oxidative DNA damage deserves further study in more occupational diseases. It is not exaggeration, oxidative DNA damage opens up a window for clinical physicians, it can broaden the breadth and depth of medical thought, there are many possibilities for flexible use in occupational medicine. Our hospital and I personally look forward to more opportunities for collaborative research.

研究者自署：胡 英華 ㊞

日中笹川医学奨学金制度（共同研究コース）
日中共同研究に関わる報告書

Supported by 日本財団 THE NIPPON FOUNDATION

作成日：2018 年 7 月 18 日

氏 名（漢字）	河井 一明	氏名（ローマ字）	KAWAI KAZUAKI
所属機関・部署・役職	産業医科大学・産業生態科学研究所 職業性腫瘍学・教授		
研究テーマ	炎症モデルラットを用いた酸化的 DNA 損傷の測定		
中国側共同研究者 氏名と研究者番号	胡 英華 K4019	中国側共同研究者 所属機関	黒竜江省第二病院

本共同研究の①研究目的の達成度、②将来性、③今後共同研究の展望や予定について述べて下さい。

【達成度】

発がん性物質の多くは、DNA に損傷を引き起こし、細胞をがん化させる。なかでも、活性酸素による DNA 損傷は、がんを始めとする多くの生活習慣病の原因とされている。そこで、生体の酸化的 DNA 損傷を正確に測定することは、発がん物質の適切なリスク評価につながる。本研究では、代表的な酸化的 DNA 損傷である 8-ヒドロキシデオキシグアノシン（8-OHdG）を精度高く測定することを目的とした。多くの疾病に繋がる炎症は、酸化的損傷と密接に関係していることから、炎症モデルラットの様々な臓器について、酸化的 DNA 損傷を測定した。当初予定した全ての臓器について測定結果が得られ、これまでに報告が無かった臓器の知見も得ることができた。また、測定値については、以前の報告に比べて低い値が得られており、測定操作に伴うアーチファクトが低減できたと考える。よって、本研究目的は、ほぼ達成できた。

【将来性】

8-OHdG については、これまでに多くの報告がされているが、測定方法の違いなどにより、測定値が異なる事が問題とされてきた。当研究室では、8-OHdG の精度の高い測定方法について検討しており、今回、炎症モデルラットを用いた測定において、共同研究者である胡氏自身が精度の高い測定を行うことができた。酸化ストレスが原因となっている疾病は、生活習慣病に限らず多く見られることから、酸化的損傷をより正確に測定することで、疾病の原因解明や予防に繋がることが期待される。

【今後の展望】

胡氏が生体の酸化ストレスを測定し評価する分析手法を身につけたことで、所属する黒竜江省第二病院において、様々な疾病の発症メカニズムの解明や治療、さらには予防対策に応用できると考える。また、共同研究で得た知見は、関連分野の情報を収集、整理、活用する際にも役立てられる。さらに、黒竜江省第二病院では、職業性疾病の治療、予防、研究に注目して取り組みを進めており、今回の共同研究において DNA の酸化損傷を測定して得た成果は、しばしば問題となる職業性発がんの分野においても、DNA 損傷解析による発がん性物質のリスクアセスメントに活用できる可能性が高い。今後、具体的な事例について共同研究を行う機会を探りたい。

日本側共同研究者自署：河井 一明 ㊞

日中笹川医学奨学金制度（共同研究コース）研究報告書

Supported by 日本財団 THE NIPPON FOUNDATION

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第 40 期　研究者番号(研究者编号)：＿＿＿＿　作成日(书写日期)：2018 年 9 月 17 日

氏　名 (姓名)	Zhaowei Meng	性別 (性别)	Male	生年月日 (出生日期)	1977-11-2

研 究 テーマ (研 究 题 目)	Genetic mutation for ^{131}I refractory papillary thyroid cancer
研究期間(来日～帰国まで) (来日起至回国的研究起止时间)	2018 年 8 月 3 日 ～2018 年 10 月 29 日
在日共同研究機関・部署 (在日共同研究单位及部门)	Department of Radiation Medical Sciences, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan
共同研究者氏名・役職 (共同研究者姓名/职务)	Norisato Mitsutake, Shunichi Yamashita
学会参加について (关于在日期间参加学会)	有り(有参加) ☐　なし(没有参加) ☑ ※参加有の方は下記の欄をご記入下さい。(有参加学会者，请继续填写如下内容)
一般参加 (普通参加)	学会名称：
一般参加 (普通参加)	学会名称：
発表有り (有发表)	学会名称： 発表テーマ(发表题目)：
発表有り (有发表)	学会名称： 発表テーマ(发表题目)：
論文発表について (关于在日期间论文发表)	発表有/投稿中(已发表或投稿中) ☐　発表なし(没有发表) ☑ ※既にもしくは発表予定有りの方は下記の欄をご記入下さい。 (已发表或有论文投稿中的，请填写如下内容，并另外单独附上全部论文内容的复印件)
	テーマ(题目)：
	著者名(作者名)：
	雑誌名(期刊名)：
	発行年(发表年度)： 巻　号(刊卷)： ページ(页数)： インパクトファクター(影响因子)：

日本滞在中の具体的な共同研究内容についての報告
（关于在日期间就研究题目具体开展的共同研究内容的详述）

【研究目的】（研究目的）

Genetic mutation happens in papillary thyroid carcinoma (PTC). The clinically confronted radioactive iodine refractory (RAIR) harbingers poor outcome of PTC. In this research, we set out to use various statistical models to investigate the value of TERT, BRAF and RAS mutations in PTC patients (cured cases and RAIR cases). First, we aimed to determine whether genetic mutation pattern could play a significant role in distinguishing these two groups of patients. Second, we attempted to show, to what extent, genetic mutation pattern could indicate non-^{131}I-avidity type of RAIR.

【研究経過】（研究经过）

TERT promoter, BRAF and RAS mutations were examined in primary lesions of 33 RAIR cases as well as age and sex-matched 34 cured cases. Thyrotropin stimulated thyroglobulin (sTg) change, lesion ^{131}I uptake ability (calculated by using tumor-to-background ratio) and RAIR category were evaluated in the ^{131}I-refractory cases. Optimal logistic regression model, PROC logistic regression model, and generalized linear model were utilized for statistics.

【成果】（成果）

Prevalence of TERT mutation in RAIR group was 24.24% (8/33), all as C228T mutation, which was significantly higher than cured group (0 incidence). BRAF mutation showed high prevalence in both cured group (64.71%) and RAIR group (69.70%) without a significant difference. Six cases had coexisting TERT and BRAF mutations. RAS mutation happened in only 1 case in cured group (NRAS 31) and 2 cases in RAIR group (one KRAS 13, one NRAS 61). When we focused on RAIR group, we saw 100% TERT mutation (both alone and in concomitant with BRAF mutation) patients showed sTg maximum or increase situation. The double mutation conferred the decreased ^{131}I uptake and was associated with RAIR categories of weaker or absent ^{131}I uptake.

In conclusion, coexisting TERT/BRAF mutations constitutes a novel genetic pattern to indicate non-^{131}I-avdity lesions in ^{131}I-refractory PTC. Although obvious differences in TERT/BRAF or TERT mutation types between RAIR group and cured group exist, clinicopathological parameters play a more important role in this comparison setting. Future studies should have matched tumor diameter, lymph node involvement and multifocality in the two groups for understanding the role of mutational status.

【今後の論文発表予定】（今后论文发表的计划）

We plan to submit this paper in the near future to a SCI journal with IF of more than 3.

【今後の課題】（今后的课题）

There are several limitations in the study. Retrospective nature of the investigation could not prevent selection bias, and could not answer causality question. The cohort is small, subgrouping made statistical power became even weaker. Some other genetic mutation pattern is not measured such as RET/PTC, AKAP9/BRAF, but these mutations happens even much rarer. For the comparison between cured patients and RAIR patients, future studies should have matched tumor diameter, N1b LN involvement and multifocality in the two groups in order to understand the role of mutational status in identifying RAIR in this comparison setting.

本共同研究の①研究目的の達成度、②将来性、③帰国後の展開予定について述べて下さい。
（请就本次合作研究题目的①研究目的的达标情况、②未来的发展可能性、③您回国后今后的合作研究的规划打算作一论述）

【達成度】（达标情况）

In this project, bilateral cooperation is the basis for the outcome in the following ways.

1) A total of 33 RAIR cases as well as age-matched and sex-matched 34 cured cases were retrospectively retrieved from database archive in Department of Nuclear Medicine of Tianjin Medical University General Hospital. The patients underwent thyroidectomy in 6 hospitals in Tianjin Municipality from the year of 2003 to 2017, then they were referred to our department for ^{131}I therapy. Intact database were available for all recruited cases. This part is made possible from the Chinese institute.

2) Formalin-fixed paraffin-embedded primary lesion samples were cut into 10-μm thickness slices. By using a QIAamp DNA mini kit, according to the manufacturer's protocols, DNA was extracted from the slices. Then, mutations of TERT promoter (C228T and C250T), BRAF (exon 15), and RAS (HRAS, KRAS and NRAS at codons 12, 13 and 61 respectively) were examined by direct DNA sequencing. Afterwards, PCR products were treated with ExoSAP-IT PCR clean-up reagent. And finally, sequencing was conducted with Big Dye Terminator sequencing kit version 3.1 on an ABI3730 automated sequencer. We included negative controls in every PCR to ensure contamination-free amplifications. This part is made possible from the Japanese institute.

3) Serum parameter evaluation and semi-quantitative imaging analysis were conducted in the Chinese institute. Thyrotropin stimulated Tg (sTg) was used as the serological marker for evaluating the response to ^{131}I therapy. After a series of at least 2 course ^{131}I therapies, we categorized sTg into three responsive groups, sTg max group (higher than Tg measurable maximum calibration value of 300 ng/ml, increase to the maximum value, or increase more than 20%), sTg decrease group (decrease more than 20%) and sTg stable group (-20% to +20%). Post ^{131}I therapeutic whole-body scan and SPECT/CT scan were performed in all cases. Semi-quantitative analysis of ^{131}I uptake in metastatic foci of RAIR group was conducted by using SPECT/CT region-of-interest (ROI) software. Briefly, ROIs were drawn around target lesions and normal frontal cranial bone separately, the mean count of each ROI was determined, and then tumor-to-background (T/B) ratio was calculated. For all of the 33 RAIR cases, we calculated two T/B ratios, one after the first ^{131}I therapy (T/B1), and another after the subsequent ^{131}I therapy (T/B2), when the residual thyroid tissue had been ablated.

4) Statistical analysis was performed in the Japanese institute. Univariate Mann-Whitney and Fisher's exact test (or Fisher-Freeman-Halton test) were applied to continuous variables or frequencies, respectively, for comparison across the subgroups. Correlation analysis and Wilcoxon signed rank test were used to compare ^{131}I uptake in sequential treatments. Multivariate logistic regression models were used to identify variables independently associating with outcomes. Analyses with very small numbers of outcomes (< 5 per cell) or when quasi-complete separation was observed were conducted using exact logistic regression. Generalized linear model was used to analyze ^{131}I uptake on a continuous scale; for this purpose, the variable was Box-Cox transformed to ensure the residuals were normally distributed; the Type III sums of squares approach was employed to account for the effects of multiple variables in the model. Non-automatic optimization of the models, starting from the full model, was performed using Akaike information criteria. Once the most appropriate model was determined, the maximum likelihood estimates of the respective parameters and their Wald-type 95% confidence intervals were calculated.

【将来性】（未来的可能性）

We had some reliable findings but also some unsatisfactory results.

1) Good finding #1. Since T/B1 and T/B2 were strongly correlated (Pearson's r=0.933, P<0.001), we performed multivariate analysis for T/B1 only. The optimal model indicated that tumors with TERT/BRAF double mutation could be expected to display a T/B ratio decreased on average by 3.83

(P<0.001), and by 2.60 in case of TERT only mutation (P=0.016). Central LN metastasis would also decrease the T/B ratio on average by 1.60 (P=0.004).
2) Good finding #2. Two types of multivariate analysis were performed. First, we determined factors distinguishing metastases with no ^{131}I uptake (RAIR category I) or with weaker uptake (RAIR category II) from those displaying avid uptake (RAIR category III). Both TERT/BRAF double mutation (OR=32.55, P=0.008) or TERT mutation only (OR=24.45, P=0.020) were significantly associated with decreased and/or absent uptake. Second, we contrasted metastases with no ^{131}I uptake (RAIR category I) against those displaying weaker (RAIR category II) or avid uptake (RAIR category III). The absence of ^{131}I uptake was strongly associated with TERT/BRAF double mutation (OR=119.71, P=0.0002) while the effect of TERT mutation only was non-significant (P=0.127).
3) Unsatisfactory finding. We found the prevalence of TERT mutation in RAIR group was 24.24% (8/33), all of which showed C228T mutation patterns. TERT mutation could demonstrate a significant difference between RAIR group and cured group (P=0.002), the latter showed 0 mutation prevalence. As for BRAF mutation, both groups showed high prevalence (cured group 64.71%, RAIR group 69.70%). BRAF did not display a discriminative value for refractoriness. However, in the scenario of both TERT/BRAF mutation, a significant difference retained (P=0.011). RAS mutation happened in only 1 case in cured group (at NRAS codon 31) and 2 cases in RAIR group (one at KRAS codon 13, the other at NRAS codon 61), all of whom were follicular subtype. Yet, RAS was not feasible for differentiation (P=0.614). On multivariate analysis, only the greater tumor diameter (OR=5.95, 95%CI 1.91-18.53, P=0.002) and N1b LN status (OR=4.84, 95%CI 1.11-21.11, P=0.036) were independently associated with RAIR cancers. Despite the differences in mutational characteristics, clinicopathological parameters appeared to be the strongest predictors of the latter. Therefore, for the comparison between cured patients and RAIR patients, future studies should have matched tumor diameter, N1b LN involvement and multifocality in the two groups in order to understand the role of mutational status in identifying RAIR in this comparison setting.

【帰国後共同研究の展開予定】（回国后的合作规划）
Because there are limitations of the current study. In the future, after I return back to China, collaborative study will continue to tackle the limitations.
1) Limitation No. 1 in the study. Retrospective nature of the investigation could not prevent selection bias, and could not answer causality question. We will plan a prospective study in the future.
2) Limitation No. 2 in the study. The cohort is small, subgrouping made statistical power became even weaker. We will include more patients in the study.
3) Limitation No. 3 in the study. Some other genetic mutation pattern is not measured such as RET/PTC, AKAP9/BRAF, but these mutations happens even much rarer. We will measure as many of these genes as possible in the future study plan.
4) Limitation No. 4 in the study. For the comparison between cured patients and RAIR patients, future studies should have matched tumor diameter, N1b LN involvement and multifocality in the two groups in order to understand the role of mutational status in identifying RAIR in this comparison setting. This is maybe the most important thing we will do, that is to plan a much more matched two groups of patients in the future. Only in this way, may we analyze the impact of mutational status under this comparison setting.

研究者自署：孟召伟 ㊞

日中笹川医学奨学金制度（共同研究コース）
日中共同研究に関わる報告書

Supported by 日本財団 THE NIPPON FOUNDATION

作成日：2018 年 10 月 25 日

氏 名（漢字）	光武　範吏	氏名（ローマ字）	MITSUTAKE, Norisato
所属機関・部署・役職	長崎大学・原爆後障害医療研究所・放射線災害医療学		
研究テーマ	甲状腺乳頭癌の放射線抵抗性に関連する遺伝子変異		
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本共同研究の①研究目的の達成度、②将来性、③今後共同研究の展望や予定について述べて下さい。

【達成度】

本研究の目的は、甲状腺乳頭癌において放射性ヨード治療への抵抗性と関連する遺伝子異常を発見することであった。放射性ヨード治療抵抗性となった腫瘍の原発巣、年齢・性別をマッチさせた治療に良好な反応を見せた症例の原発巣より DNA を抽出し、長崎大学で確立した手法を用い、*RAS*、*BRAF*、*TERT* プロモーターにおける遺伝子変異を解析した。さらに、種々の臨床病理学的データも収集し、様々な統計学的モデルを駆使して、遺伝子変異を含め、放射線治療抵抗性に影響を及ぼす因子の同定を行った。その結果、*TERT* プロモーター変異は、放射線治療抵抗性に非常に高い相関を示したが、独立した抵抗性予測因子とは言えず、さらなる解析を行う必要が示唆された。しかし、放射線治療抵抗性群において、*TERT* プロモーター変異は放射性ヨード取り込みと関連があり、この点では独立した予測因子であることが明らかになった。以上をまとめた論文を現在国際英文誌に投稿中である。わずか 3 ヵ月で全ての解析から論文投稿まで行うことができ、本共同研究の達成度はかなり高いと言えよう。

【将来性】

本共同研究によって、放射性ヨード治療の効果と遺伝子変異の関連が明らかになり、これは臨床的にも非常に重要な知見である。今後、さらに研究を進め、より詳細な解析結果を出すことができれば、治療方針決定にも影響を与え、患者ひとりひとりに最適なテーラーメード医療を展開する基盤とすることができるだろう。中国では放射性ヨード治療を受ける患者数は非常に多く、多数の症例を用いた研究を短期間に終わらせることができる可能性がある。本共同研究は今後重要になってくるものと思われる。

【今後の展望】

今回の共同研究では、いくつかの今後解決すべき問題点も明らかになった。まず、*TERT* プロモーター変異は、腫瘍径や年齢と非常に強い相関があり、それらを考慮した試料を用いた研究を行う必要があることが分かった。また、今回の研究では、これまで収集された試料を用いた後ろ向き研究であり、様々なバイアスが含まれている可能性がある。さらに、古い試料からは十分な品質の核酸が抽出されないものもあった。これらの問題点を克服し、より精度の高い研究成果を挙げるために、今回の解析を行った経験を十分に活かした試料収集ネットワークの構築が重要になってくるだろう。今後も緊密な連絡体制を維持しつつ、共同研究を継続していく予定である。

日本側共同研究者自署：光武範吏　印

日中笹川医学奨学金制度

第４０期（学位取得コース）

中　間　報　告　書

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

Supported by 日本財団 THE NIPPON FOUNDATION

第40期　　研究者番号：G4001　　作成日：2019年3月7日

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研究テーマ	中国におけるマダニとマダニ媒介感染症の疫学調査と有効な駆除法の開発 Epidemiological study on ticks and tick-borne diseases in China and establishment of their control measures				
専攻種別	論文博士	☑	課程博士	□	

1．研究概要（1）

1）目的（Goal）

Ticks, as one group of hematophagous arthropods, can transmit various pathogens to humans and animals, with great health and veterinary significance. *Babesia microti* is one of human pathogenic agents, and has been reported in tick-borne disease endemic region in southwestern and northeastern China. Tick population was systematically surveyed, and tick-borne pathogens in vectors and host animals were detected in this study. We selected predominant ticks to study interaction of pathogens and ticks, and attempted to screen proteins derived from ticks for suppression of tick-borne pathogen transmission to tick vectors and host animals.

2）戦略（Approach）

Sampling methods\surveillance of tick populations and detection of tick-borne pathogens in tick vectors and hosts\construction of SSH library\screening proteins of great importance in pathogen transmission\evaluation of some proteins in pathogen transmission blocking (potential vaccine for tick-borne diseases).

3）材料と方法（Materials and methods）

Tick population characterization

Sample collection

We planned 12 investigation sites in Jiangxi province, southeastern China and one investigation site in Heilongjiang province, northeastern China. For each investigation site in Jiangxi province, tick collection was performed by flagging vegetation and picking up ticks from host animals. For the investigation site in Heilongjiang, the ticks were collected by flagging vegetation.

Tick identification

Ticks were identified to species level according to taxonomic keys compiled by Sun Yi and Xu Rongman and molecular verification with DNA marker COI.

Ticks in habitats

Tick distribution patterns in habitats were expressed as numbers of ticks at different developmental stages.

Ticks on host animals

Infestation rate was calculated as infested hosts divided by total ticks for specific tick species, and difference in infestation rate was evaluated by Chi square. P>0.05 indicates that difference in infestation rate of host animals is significant. Ticks/hosts was denoted as tick density. We also calculated tick composition as specific ticks in total ticks.

Tick-borne pathogens in ticks

Tick population

Ticks were identified to species level and developmental stages.

Tick-borne pathogens in ticks from Jiangxi

Tick-borne pathogens were detected in ticks from Jiangxi by amplification of genes of tick-borne pathogens . Amplicons were sent to Shanghai Sangon Biotechnology for sequencing, and pathogen verification was conducted by alignment with candidate sequence deposited in GenBank.

Tick-borne pathogens in ticks from Heilongjiang

Tick-borne pathogens of babesiosis were tested by ticks collected in tick-borne disease endemic regions of northeastern China infesting laboratory rodents. Every mouse was loaded with 10 ticks and then checked for the presence of Babesia by blood smear stained with Giemsa and PCR followed by sequencing each day 5 days post tick attachment. The samples positive for Babesia were further characterized by in vivo serial passages, in vitro cultivation, blood smears, PCR detection followed by sequencing, transmission electron microscope (TEM) and scanning electron microscope (SEM).

1．研究概要（2）

Tick-borne pathogens in host animals

Blood samples from host animals were collected in Jiangxi and used to detect the presence of tick-borne pathogens. Genomic DNA was amplified by pair primers. Amplified products were sent to Shanghai Sangon Biotechnology for sequencing. Trimmed DNA sequences were aligned with candidate sequences deposited in GenBank of NCBI and subsequently pathogen species or genera or family were checked.

Interface of *Haemaphysalis longicornis* and *Babesia microti*

Haemaphysalis langicarnis is frequently found in mainland China from south to north and now reported as predominant tick species in many regions, including Henan, Hubei, Shandong and Jiangxi. Babesia species are also prevalent in host animals and tick vectors from China, and we found *Babesia vogeli* and *Babesia gibsoni* in dog hosts and *B. vogeli* in *Haemaphysalis flava* from southeastern China. Meanwhile, we also determined at least three Babesia species in *Ixodes persulcatus* obtained by flagging vegetation from northeastern China. Here we used Chinese predominant tick *H. longicornis* and human pathogenic agent *Babesia microti* Gray strain to establish tick-pathogen interaction model although *H. longicarnis* is not efficient vector of *B. microti* Gray strain.

4）実験結果（Results）

Tick populations in Jiangxi

Fifteen tick species were found, including *H . longicornis , Rhipicephalus sanguineus sensu lato , Haemaphysalis yeni , Haemaphysalis kitaokai, Ixodes sinensis, Dermacentar auratus, Haemaphysalis campanulata, H. flava, Haemaphysalis doenitzi, Haemaphysalis hystricis, Rhipicephalus haemaphysaloides, Rhipicephalus microplus, Ixodes granulatus, Amblyomma testudinarium* and *Ixodes acuminatus. H. longicornis* was the most frequently collected species and widely distributed tick species of the total collection ticks (in 11 sampling sites), and had a broad host range.

Tick-borne pathogens in Jiangxi

Tick-borne pathogens in ticks included *Borrelia yangtzensis* in *I. granulatus, Rickettsia raoultii* or *Rickettsia slovaca* related genospecies in *H. longicornis* and *H. flava, Hepatozoon canis* or *Hepatozoaan felis* related genospecies in *H. longicornis,* and *B. vageli* in *H. flava.* We also found *Mycoplasma haemacanis, B. gibsoni* and *B. vogeli* in dogs and *Anaplasma phagocytophilum* in rodents.

Tick-borne babesias in Heilongjiang

Ixodes persulcatus ticks were collected from tick-borne disease endemic region and used for the detection of tick-borne babesias. Consequently, three Babesia species including *Babesia bigemina, Babesia divergens* and *Babesia venatorum* were detected in engorged ticks on SCID mice, and two of them were isolated via tick bite on SCID mice and culturing in the human and mouse erythrocytes, namely *B. divergens* and *B. venatorum.*

5）考察（Discussion）

Zoogeographically, China is divided into Palaearctic and Oriental Realm, and has abundant tick species which form approximately 1/8 of tick species worldwide [1]. *H. longicornis* is a predominant tick species in many regions of China, including Niaoning, Henan, Hubei, Shandong and Jiangxi[2-6], and can vector variety of tick-borne pathogens like *Rickettsia, Babesia, Borrelia* and some human pathogenic viruses [7,8]. *Babesia* species are a group of tick-borne protozoa prevalent in China, found in southwestern, central and northeastern China [7]. Here, we detected *B. vogeli* and *B. gibsani* in Jiangxi [9-10], and isolated *B. divergens* and *B. venatorum* from *I. persulcatus* from Helongjiang. *B. microti* and its related genospecies are another Babesia species frequently determined in China, ranging from north to south and from west to east [7,11]. In future research, we will exploit interface of *H. longicornis* and *B. microti* Gray strain, and screen important tick proteins for suppression of tick-borne pathogen transmission in hosts and ticks.

6）参考文献（References）

[1] Chen Z, Yang XJ, Bu FJ, et al. Ticks (Acari: Ixodoidea: Argasidae, Ixodidae) of China. Experimental and Applied Acarology, 2010, 51: 393-404.

[2] Zheng WQ, Xuan XN, Fu RL, et al. Preliminary investigation of ixodid ticks in Jiangxi Province of Eastern China. Experimental and Applied Acarology, 2019, 77: 93–104.

[3] Zhang FS, Liu XP, Gao Y, et al. Investigation on the distribution of tick population in Benxi city. Chinese Journal of Hygienic Insecticide and Equipment, 2015, 21:515-516, 519.

[4] Zhang QZ, Luo HL, Li XW, et al. Survey on ticks and pathogens in the serum of its host in Xinyang city, Henan province. Journal of Henan Normal University (Natural Science Edition), 2016, 44:126-130.

[5] Wu TY, Wang W, Chen SB, et al. Preliminary survey of ticks and tick-borne pathogens in Tianjin, China. Chinese Journal of Vector Biology and Control, 2013, 24:246-248.

[6] Zhang LY, Li J, Zhan F, et al. Investigation on the *Haemaphysalis longicornis* infected with *Anaplasma phagocytophilum* in Hubei province, China. Chinese Journal of Zoonses, 2010, 26:1148-1150.

[7] Fang LQ, Liu K, Li XL, et al. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infectious Diseases, 2015, 15: 1467-1479.

[8] Wu XB, Na RH, Wei SS, et al. Distribution of tick-borne diseases in China. Parasit Vectors, 2013, 6: 119.

[9] Zheng WQ, Xuan XN, Fu RL, et al. Tick-borne pathogens in ixodid ticks from Poyang Lake region, southeastern China. Korean Journal of Parasitology, 2018, 56:589-596.

[10] Zheng W, Liu M, Moumouni PF, et al. First molecular detection of tick-borne pathogens in dogs from Jiangxi, China. Journal of Veterinary Medical Science, 2017, 79: 248-254.

[11] Zhou X, Li SG, Chen SB, et al. Co-infections with *Babesia microti* and *Plasmodium* parasites along the China–Myanmar border. Infectious Diseases of Poverty, 2013; 2: 24.

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title	First Molecular evidence of *Anaplasma phagocytophilum* in rodent populations of Nanchang, China					
掲載誌名 Published journal	Japanese Journal of Infectious Diseases					
	2018 年 2 月	71 巻（号）	129 頁 ～ 133 頁	言語 Language	English	
第 1 著者名 First author	Weiqing Zheng	第 2 著者名 Second author	Yangqing Liu	第 3 著者名 Third author	Huiying Tao	
その他著者名 Other authors	Zifen Li, Xuenan Xuan, Xiaoqing Liu, Paul Franck Adjou Moumouni, Yayun Wu, Wenqing Liu, Haiying Chen					
論文名 2 Title	Tick-borne pathogens in Ixodid ticks from Poyang Lake Region, Southeastern China					
掲載誌名 Published journal	Korean Journal of Parasitology					
	2018 年 12 月	56（6） 巻（号）	589 頁 ～ 596 頁	言語 Language	English	
第 1 著者名 First author	Weiqing Zheng	第 2 著者名 Second author	Xuenan Xuan	第 3 著者名 Third author	Renlong Fu	
その他著者名 Other authors	Huiying Tao, Yangqing Liu,Xiaoqing Liu, Dongmei Li, Hongmei Ma, Haiying Chen					
論文名 3 Title	Preliminary investigation of ixodid ticks in Jiangxi Province of Eastern China					
掲載誌名 Published journal	Experimental and Applied Acarology					
	2019 年 1 月	77(1) 巻（号）	93 頁 ～ 104 頁	言語 Language	English	
第 1 著者名 First author	Weiqing Zheng	第 2 著者名 Second author	Xuenan Xuan	第 3 著者名 Third author	Renlong Fu	
その他著者名 Other authors	Huiying Tao, Rongman Xu, Yangqing Liu,Xiaoqing Liu, Jiafu Jiang, Haixia Wu, Hongmei Ma, Yi Sun, Haiying Chen					
論文名 4 Title						
掲載誌名 Published journal						
	年 月	巻（号）	頁 ～ 頁	言語 Language		
第 1 著者名 First author		第 2 著者名 Second author		第 3 著者名 Third author		
その他著者名 Other authors						
論文名 5 Title						
掲載誌名 Published journal						
	年 月	巻（号）	頁 ～ 頁	言語 Language		
第 1 著者名 First author		第 2 著者名 Second author		第 3 著者名 Third author		
その他著者名 Other authors						

3．学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載してくた

※Describe your presentation as the principal presenter in major academic meetings including general meetings or international meetings.

学会名 Conference	National Research Center for Protozoan Diseases seminar		
演題 Topic	The role of several tick genes in acquisition of Babesia microti in Haemaphysalis longicornis ticks		
開催日 date	2018 年 7 月 26 日	開催地 venue	Obihiro, Hokkaido
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poster	言語 Language	☐ 日本語 ☑ 英語 ☐ 中国語
共同演者名 Co-presenter			
学会名 Conference			
演題 Topic			
開催日 date	年 月 日	開催地 venue	
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Poster	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語
共同演者名 Co-presenter			
学会名 Conference			
演題 Topic			
開催日 date	年 月 日	開催地 venue	
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Poster	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語
共同演者名 Co-presenter			
学会名 Conference			
演題 Topic			
開催日 date	年 月 日	開催地 venue	
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Poster	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語
共同演者名 Co-presenter			

4．受賞（研究業績）Award (Research achievement)

名称 Award name				
国名 Country name		受賞年 Year of award	年 月	
名称 Award name				
国名 Country name		受賞年 Year of award	年 月	

5. 本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	□ 有 ■ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円
受給実績 Receipt record	□ 有 ■ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

6. 他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	□ 有 ■ 無
助成機関名称 Funding agency	
奨学金名称 Scholarship name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

7. 研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。Attach a copy of the article described below

報道発表 Press release	□ 有 ■ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞 ・雑誌 ・Web site ・記者発表 ・その他（ ）		
発表タイトル Released title			

8. 本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled application	□ 有 ■ 無	出願国 Application country	
出願内容（概要） Application contents			

9. その他 Others

指導責任者（署名） 玄 学南 印

5．本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	Jiangxi Provincial Department of Science and Techology
助成金名称 Grant name	Key research project of Jiangxi province (20161BBG70005)
受給期間 Supported period	2016 年 6 月 ～ 2019 年 6 月
受給額 Amount received	830,000 円
受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	Health and Family Planning Commission of Jiangxi Province
助成金名称 Grant name	Program for Science and Technology of Health and Family Planning Commission of Jiangxi Province(20162007)
受給期間 Supported period	2016 年 1 月 ～ 2018 年 12 月
受給額 Amount received	66,484 円
受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	Nanchang Science and Technology Bureau
助成金名称 Grant name	Hong Scientific Research Program [2016](No. 96 Item 77)
受給期間 Supported period	2016 年 1 月 ～ 2018 年 1 月
受給額 Amount received	0 円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

日中笹川医学奨学金制度(学位取得コース)中間評価書
論文博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　研究者番号：G4001　作成日　：2019 年 3 月 13 日

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研究テーマ	中国におけるマダニとマダニ媒介感染症の疫学調査と有効な駆除法の開発				
専攻種別	■ 論文博士		□ 課程博士		

研究者評価（指導教官記入欄）

成績状況	良	取得単位数 取得単位数／取得すべき単位総数
学生本人が行った研究の概要	当該研究者はこの一年間、中国（江西省、黒竜江省）におけるマダニとマダニ媒介感染症に関する疫学調査を実施し、下記のような成果が得られた。 1）江西省に分布しているマダニを採集し、種の同定を行ったところ、計 15 種類のマダニ種を特定した。 2）江西省に分布しているマダニおよび動物からバベシア属、ボレリア属、リケッチア属、アナプラズマ属などマダニ媒介病原体を検出し、これらの病原体が当該地域において、人や動物に健康被害を与える可能性を示唆した。 3）黒竜江省から採集したマダニを実験動物に吸血させた後に、病原体の検査を行ったところ、３種類のバベシア属原虫の分離に成功した。	
総合評価	【良かった点】 当該研究者の所属機関の所在地の周辺地域における疫学調査の結果は、今後マダニとマダニ媒介感染症対策を講ずる上で貴重な基礎データになるものである。	
	【改善すべき点】 マダニとマダニ媒介病原体について、もっと幅広い情報収集（世界的分布と被害状況）などに努めて欲しい。	
	【今後の展望】 これまでに得られたデータに基づいて、マダニとマダニ媒介感染症対策の確立が期待される。	
学位取得見込	２０１９年度中の取得は厳しい状況であるが、２０２０年度以降の取得は十分期待できる。	
評価者（指導教官名）	玄 学南 ㊞	

Jpn. J. Infect. Dis., 71, 129–133, 2018

Original Article

First Molecular Evidence of *Anaplasma phagocytophilum* in Rodent Populations of Nanchang, China

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SUMMARY: In this study, systematic surveillance of rodent populations in Nanchang of China and determination of *Anaplasma phagocytophilum* infection in rodents were performed. Between 2011 and 2015, 110,084 rodent snap traps were set in 4 counties and in the city center of Nanchang, China. Finally, 942 rodents were captured, with a relative density of 0.86%. The densities varied considerably by geographical area with Anyi being the most rodent-infested County. Frequently captured rodents were sewer rats (*Rattus norvegicus*), house mice (*Mus musculus*), and *Rattus flavipectus*. The *Anaplasma* genera were investigated by PCR in 19 live rodents trapped by welded cages in Anyi, 6 rodents were assessed as positive based on amplification of 16S rRNA. Sequence analysis revealed 3 variants of *A. phagocytophilum* in Nanchang. PCR analysis of the *gltA* (citrate synthase) gene found 1 sample that was positive for *A. phagocytophilum* infection. The sequence of *A. phagocytophilum gltA* gene formed a clade with and showed 99% identity to *A. phagocytophilum* that has been previously described in rodents from South-Eastern China. Taken together, our research indicated that commensal rodents are potential hosts for *A. phagocytophilum* and controlling the rodent population may facilitate subsequent prevention of human granulocytic anaplasmosis in Nanchang, China, in the future.

INTRODUCTION

Rodents are important destructive animals globally that compete with humans for food, particularly through pre-harvest damage to rice crops. Some rodents occur commensally in human residential areas, easily contaminating the human environment by the production of metabolic excrement, faeces, and urine. Important pathogens like hemorrhagic fever with renal syndrome virus can be transmitted directly to humans by contact with objects and inhalation of air contaminated by rodents. Other pathogens, like *Borrelia* spirochetes, can indirectly infect humans through the bites caused by ectoparasites, such as lice, fleas, and ticks on the rodents. The research mentioned above was reviewed and documented in previous works (1,2).

Human granulocytic anaplasmosis (HGA) is considered a rodent-borne disease caused by *Anaplasma phagocytophilum*, which is an emerging obligate intercellular bacterial pathogen (3). HGA is increasingly recognized as an important and frequent cause of fever worldwide (4). Anaplasmosis was first recognized as a disease of humans in the United States in the mid-1990s. Cases of anaplasmosis have generally increased from 350 cases in 2000 to 1,163 cases in 2009. The number of reported cases increased by 52% between 2009 and 2010 (5). By the end of 2015, more than 100 HGA cases had been reported in Mainland China, covering the north and south of the Yangtze River. The cases were mainly distributed in Shandong and Beijing (6). In 2010, an HGA case was reported in a healthy young man from Nanchang of Jiangxi Province, China. The patient noticed an unknown arthropod bite one day before the initiation of fever, and died 10 days later (unpublished data). Clinical manifestations in humans range from mild self-limiting febrile illness to fatal infections. The most frequent manifestations in patients are non-specific influenza-like symptoms with fever, headache, myalgias, and malaise. Few patients have arthralgia or involvement of the gastrointestinal tract, respiratory tract, liver, or the central nervous system (4).

A. phagocytophilum appears to be a generalist, infecting a wide range of animals, such as humans, domestic animals, and wildlife, including rodents, throughout the world (7–10). Previous studies showed that various field and domestic animals are considered as important reservoirs of *A. phagocytophilum*. Roe deer play a key role in the endemic cycles of the pathogen in Germany (11). Dogs from both urban and rural habitats in Brazil were found to be positive for *A. phagocytophilum* (12). In addition, goats and sheep were highly infected with *A. phagocytophilum* in China (9). In Nanchang, however, a previous examination could not detect the presence of *A. phagocytophilum*. In the survey, 328 dogs and goats were found to be negative for *A. phagocytophilum* infection (unpublished data), suggesting that domes-

Received June 26, 2017. Accepted November 20, 2017.
J-STAGE Advance Publication February 28, 2018.
DOI: 10.7883/yoken.JJID.2017.301
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tic animals might have a limited role as hosts for *A. phagocytophilum* in Nanchang.

Rodents are common in both urban and rural human residential areas of Nanchang city (13). Furthermore, they can cause certain rodent-borne diseases, such as hemorrhagic fever with renal syndrome and leptospirosis (12,13). However, little information on the systematic statistics of the rodent population and the presence of *A. phagocytophilum* in commensal rodents is available. The objective of this study was to survey a wide range of commensal rodent populations monthly, covering all regions of Nanchang, between the period of 2011 and 2015. The highest rodent-infested county was then selected to monitor whether *A. phagocytophilum* infection could be found in rodents.

MATERIALS AND METHODS

Commensal rodent surveillance: Rodent population densities were determined using snap traps at 58 sites in Nanchang city from 2011 to 2015. The sites were distributed in 4 counties and the city center, and were randomly selected with regard to land use and geographic location (Fig. 1A). Rodent snap traps, with fried bread sticks as bait, were set in the evening and collected in the next morning. A total of 61,554, 12,088, 12,209, 12,352, and 11,881 traps were placed in the city center, Xinjian County, Nanchang County, Anyi County, and Jinxian County, respectively. Traps were spaced at approximately 5-meter intervals in the vertical direction and at 25-meter intervals in the lateral direction. All rodents captured were measured, weighed, examined for sex and developmental condition, and, finally, morphologically identified. The rodent population density was calculated according to the following equation:

$$R(\%) = \frac{N_r}{N_e} \times 100$$

Where: R = rodent population density:
N_r = number of traps with captured rodents:
N_e = number of effective traps collected; effective traps indicating non-exciting traps and the traps with rodents.

Occurrence of *A. phagocytophilum* in commensal rodents: Live rodents were trapped using welded cages in Anyi, the county with the highest rodent density in Nanchang city. Rodent capture sites were localized in 3 residential areas near 2 brick kiln industries, and a pig breeding base without optimal hygienic conditions (Fig. 1B). Rodents were captured from late March to early April of 2016. Traps were spaced 10 m apart along lines, with bait and were checked in the morning at 24 h and 48 h after setup. Captured rodents were characterized and morphologically identified before blood collection. Blood samples were collected through incision of the femoral artery and were then transferred to EDTA-treated tubes. All samples were stored at –80°C until analyses.

Genomic DNA was extracted from a volume of 200 µl of whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendation. One hundred micro-liter eluted DNA samples (30–90 ng/µl) were harvested and stored at –30°C for subsequent PCR analysis. DNA concentrations and purities were determined with a NanoDrop Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The samples with A_{260}/A_{280} of 1.8–2.0 ratio and a concentration greater than 20 ng/µl were used in the analysis. The presence of *Anaplasma* genus DNA was examined by a semi-nested PCR assay targeting 16S rRNA as previously described (14). DNA of *A. phagocytophilum* was detected by a nested PCR based on the *gltA* (citrate synthase) gene, according to a previous description (15).

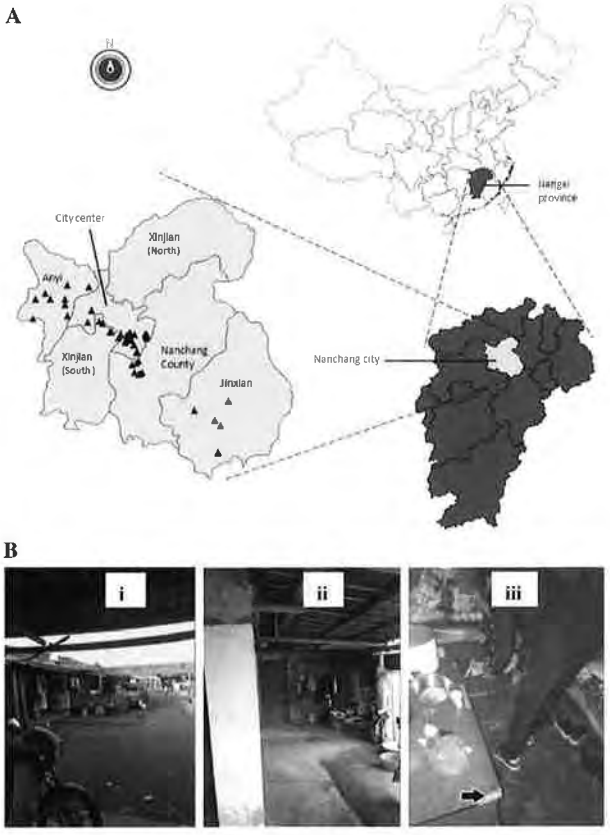

Fig. 1. (A) Investigation sites. Filled triangles on the map show 58 rodent surveillance sites. (B) Rodent capture site. (i) Full view of a residential area; (ii) Outside view of a house; (iii) Indoor view of a room, household items were in chaotic arrangement. Rodent faeces and bites in food and household items, such as tables and shoes, were observed here and there. A black arrow with white margin shows a rodent gnaw mark on a table.

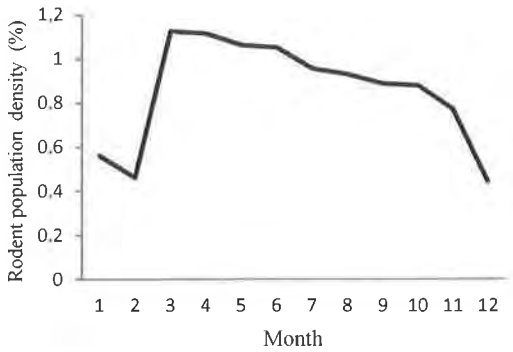

Fig. 2. The fluctuation of rodent population density in Nanchang

Samples positive for *Anaplasma* genus (*phagocytophilum*) infection were randomly selected for cloning and sequencing of amplicons. For each specimen, at least 5 clones confirmed positive by colony PCR were multiplied and purified using a NucleoSpin Plasmid Quick Pure (Macherey-Nagel, Düren, Germany). Sequencing reactions were performed using ABI BigDye Terminator Kit v3.1 (Applied Biosystems, Foster City, CA, USA). DNA fragment sequences were determined using an ABI PRISM 3100 DNA sequencer (Applied Biosystems). Identities and similarities of the nucleotide sequences were analyzed using BLASTN online at GenBank. The SeqMan and MEGA 6 programs were used to build the alignment of all selected sequences, and then a neighbor-joining phylogenetic tree was constructed using the MEGA 6 program. The confidence of internal branches was estimated by bootstrapping with 1,000 replications.

Statistical analysis: The results were analyzed by using the χ^2 test in IBM SPSS Statistics ver. 20 (Chicago, IL, USA). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Rodent population density in Nanchang between 2011 and 2015: Over the five-year trapping period, 944 (0.86%) rodents were caught at 58 sites in Nanchang. The overall male-to-female sex ratio was 1:1.02 (467:477), and the rodent population density did not differ significantly between sexes ($\chi^2 = 0.105$, df = 1, $P > 0.05$). However, the rodent population density was significantly higher between 2011 and 2013 (0.92% to 0.99%) than from 2014 to 2015 (0.68% to 0.73%) ($\chi^2 = 22.14$, df = 4, $P < 0.001$; Table 1). The rodent population density showed a peak in March–April, followed by a gradual decline from May to a low level in October. This was followed by a substantial decrease in density from October to the lowest level in December (Fig. 2).

Rodents captured included sewer rats (*Rattus norvegicus*), house mice (*Mus musculus*), and *Rattus flavipectus*. The most frequently captured rodents were sewer rats, accounting for 34.75% (328 individuals); house mice consisted of 33.47% (316 individuals). 28.07% (265 rodents) of captures were *R. flavipectus*. Some rarely seen small wild mammals were also caught around residences during the trapping period, for example, rodents (*Apodemus agrarius* and *Rattus losea*) and the insectivore *Suncus murinus*. Rodent populations were at the highest density (3.65%) in Anyi County of Nanchang city. Anyi County had three-fold higher rodent population density than Nanchang County, which had the second highest (0.99%) rodent population density at the county level across Nanchang city. Sewer rats in Anyi County had undergone large population increases between 2012 and 2015 and consequently became the

Table 1. Average annual rodent density and composition at the county level in Nanchang city, China, between 2011 and 2015

Location	Species	2011	2012	2013	2014	2015	Whole period
City center	Sewer rats	0.21 (26)[1)]	0.36 (44)	0.36 (45)	0.17 (21)	0.24 (30)	0.27 (166)
	Rattus flavipectus	0.12 (15)	0.04 (5)	0.07 (9)	0.05 (6)	0.12 (15)	0.08 (50)
	House mice	0.08 (10)	0.03 (4)	0.04 (5)	0.03 (4)	0.05 (6)	0.05 (29)
	Other	0.04 (5)	0.02 (2)	0.02 (2)	0.00 (0)	0.00 (0)	0.01 (9)
	subtotal	0.46 (56)	0.45 (55)	0.49 (61)	0.26 (31)	0.41 (51)	0.41 (254)
Xinjian County	Sewer rats	0.16 (4)	0.28 (7)	0.12 (3)	0.13 (3)	0.20 (5)	0.18 (22)
	Rattus flavipectus	0.21 (5)	0.16 (4)	0.29 (7)	0.22 (5)	0.16 (4)	0.21 (25)
	House mice	0.45 (11)	0.52 (13)	0.41 (10)	0.04 (1)	0.16 (4)	0.32 (39)
	Other	0.29 (7)	0.00 (0)	0.00 (0)	0.09 (2)	0.04 (1)	0.08 (10)
	subtotal	1.11 (27)	0.97 (24)	0.81 (20)	0.49 (11)	0.57 (14)	0.79 (96)
Nanchang County	Sewer rats	0.16 (4)	0.28 (7)	0.04 (1)	0.00 (0)	0.13 (3)	0.12 (15)
	Rattus flavipectus	0.24 (6)	0.16 (4)	0.16 (4)	0.25 (6)	0.04 (1)	0.17 (21)
	House mice	0.73 (18)	1.19 (30)	0.83 (21)	0.25 (6)	0.39 (9)	0.69 (84)
	Other	0.00 (0)	0.00 (0)	0.00 (0)	0.04 (1)	0.00 (0)	0.01 (1)
	subtotal	1.13 (28)	1.63 (41)	1.03 (26)	0.54 (13)	0.57 (13)	0.99 (121)
Anyi County	Sewer rats	0.52 (13)	0.28 (7)	0.49 (12)	1.05 (26)	2.42 (60)	0.96 (118)
	Rattus flavipectus	1.17 (29)	2.03 (50)	1.91 (47)	0.97 (24)	0.40 (10)	1.30 (160)
	House mice	1.49 (37)	1.63 (40)	1.71 (42)	1.46 (36)	0.16 (4)	1.29 (159)
	Other	0.04 (1)	0.04 (1)	0.16 (4)	0.04 (1)	0.28 (7)	0.11 (14)
	subtotal	3.22 (80)	3.98 (98)	4.28 (105)	3.52 (87)	3.27 (81)	3.65 (451)
Jinxian County	Sewer rats	0.09 (2)	0.00 (0)	0.00 (0)	0.22 (5)	0.00 (0)	0.06 (7)
	Rattus flavipectus	0.17 (4)	0.00 (0)	0.16 (4)	0.00 (0)	0.04 (1)	0.08 (9)
	House mice	0.17 (4)	0.04 (1)	0.00 (0)	0.00 (0)	0.00 (0)	0.04 (5)
	Other	0.04 (1)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.01 (1)
	subtotal	0.47 (11)	0.04 (1)	0.16 (4)	0.22 (5)	0.04 (1)	0.19 (22)
Nanchang city	Sewer rats	0.22 (49)	0.29 (65)	0.27 (61)	0.26 (55)	0.45 (98)	0.30 (328)
	Rattus flavipectus	0.27 (59)	0.28 (63)	0.32 (71)	0.19 (41)	0.14 (31)	0.24 (265)
	House mice	0.36 (80)	0.40 (88)	0.35 (78)	0.22 (47)	0.10 (23)	0.29 (316)
	Other	0.06 (14)	0.01 (3)	0.03 (6)	0.02 (4)	0.04 (8)	0.03 (35)
	Total	0.92 (202)	0.99 (219)	0.97 (216)	0.68 (147)	0.73 (160)	0.86 (944)

[1)]: Numbers before the round brackets represent rodent density (in %), and numbers in the parentheses are the total number of species-specific rodents in different years.

dominant rodent species in 2015 (Table 1).

Occurrence of *A. phagocytophilum* in rodents: A total of 19 live rodents were captured including 17 sewer rats, 1 *R. flavipectus*, and 1 home mouse in Anyi County. The distribution of rodents according to sex, stage, and infection status is shown in Table 2. The *Anaplasma* genus was detected in 6 (31.58%) rodents based on 16S rRNA PCR, and in 1 (5.26%) rodent by *gltA* amplification.

Four sewer rats and 1 *R. flavipectus* that were positive for *Anaplasma* genus infection by nested PCR of the partial 16S rRNA sequence were selected for sequence analyses. A 335-base pair nucleotide sequence was obtained from 5 individual clones of each sample. These sequences showed 94.53–99.40% identity with the published sequences of the HGA agent (KT454992).

Table 2. Presence of *A. phagocytophilum* in live rodent captures by using wire cages

No.	Species	Sex	DS	Result
1	SR	F	adult	
2	SR	F	adult	
3	SR	M	adult	
4	SR	M	adult	++ KY024480, KX757024[1)]
5	SR	M	adult	
6	SR	F	immature	
7	HM	M	adult	
8	SR	M	adult	
9	SR	F	immature	
10	SR	F	immature	
11	SR	F	adult	+ KX757023
12	SR	M	adult	+ KX757023
13	SR	M	adult	+
14	SR	F	adult	
15	SR	M	adult	+ KX757023
16	SR	M	adult	
17	SR	F	adult	
18	SR	M	adult	
19	Rf	M	adult	+ KY024479

DS, developmental stage; SR, sewer rat; HM, house mouse; Rf, *Rattus flavipectus*; F, female; M, male; +, the rodents found to be positive for *Anaplasma phagocytophilum* based on the amplification of 16S rRNA and DNA sequencing. ++, the rodent found to be positive based on both 16S rRNA and *gltA* PCR. Letter/number combinations are accession No. of target 16S rRNA and *gltA* sequence deposited in GenBank.

1): The former is accession No. of *gltA* sequence, and the latter is accession No. of 16S rRNA.

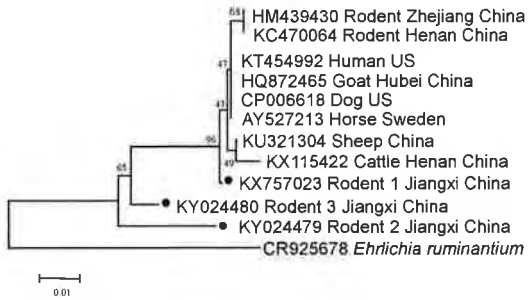

Fig. 3. Phylogenetic analysis based on *Anaplasma* genus 16S rRNA fragment. ●, sequences were obtained in this study. The *Ehrlichia ruminantium* homolog gene (CR925678) was used as the outgroup. The number of each branch was provided as bootstrap value at the node.

Sequence analysis of the 25 plasmid clones revealed 3 variants of *A. phagocytophilum*. One (KX757023) was found only in sewer rats and was highly identical to the sequence from the HGA agent, with only 2 nucleotide differences at positions of 773 and 899 and formed a clade with the sequence of the HGA agent in the phylogenetic tree (Fig. 3). In the phylogenetic tree, the other variants (KY024479 and KY024480) formed distinct branches. These variants differed from the HGA agent by 7 and 18 base-pairs, respectively. The sequences of the 3 variants obtained in the study were not 100% identical to the sequences from non-rodent hosts (HQ872465, KU321304, and KX115422), or even from rodent hosts (HM439430 and KC470064) in China. However, the fragments (HM439430 and KC470064) from rodents in China fully constructed a subclade (Fig. 3).

Five clones of the *A. phagocytophilum gltA* sequence were obtained from a sewer rat in our study. Four clones (numbered 1, 3, 4, and 5) had identical sequences to each other and differed from clone 2 by a single nucleotide mutation. Here, we used clone 1 to represent *A. phagocytophilum gltA* sequence (KX757024) extracted from the sewer rat. Our sequence was well separated from other *A. phagocytophilum* corresponding sequences from dog (KP861637) and sheep (KP861639) hosts, and tick (KP861638 and KP276595) vectors in the phylogenetic tree. However, the sequence (KX757024) shared a clade with *A. phagocytophilum* previously described in rodents from south-eastern China (DQ458809) and showed 99% identity to each other (Fig. 4).

DISCUSSION

Rodents are suspected to act as natural reservoirs for *A. phagocytophilum*. The prevalence of *A. phagocytophilum* was detected in *Apodemus sylvaticus*, *Apodemus flavicollis*, and *Myodes glareolus* in France (7). The first wild animals observed to be positive for *A. phagocytophilum* in east China were *Apodemus peninsulae*, *A. agrarius*, and *Eutamias sibiricus* (15). *A. agrarius* was subsequently found to be infected with *A. phagocytophilum* in west China (9). This evidence appeared to support the role of the genus *Apodemus* as reservoirs in the natural life cycle of *A. phagocytophilum*.

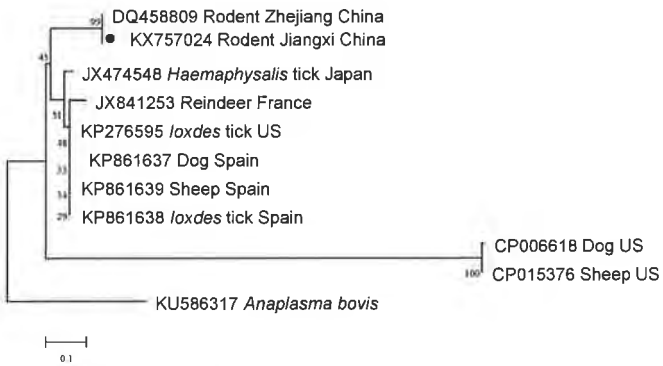

Fig. 4. Phylogenetic analysis based on *A. phagocytophlium gltA* fragment. ●, sequence was obtained in this study. The *Anaplasma bovis* homolog gene (KU586317) was used as the outgroup. The number of each branch was provided as bootstrap value at the node.

The fragment of 16S rRNA from *A. phagocytophilum* was detected in blood samples from 6 rodents by semi-nested PCR and DNA sequence analysis. However, the *gltA* gene of *A. phagocytophilum* was determined in only 1 rodent. The difference in detection rates of the same samples was likely explained by the failure of our primers to bind to highly variable regions of *gltA* gene. A recent study in France (7) showed that 6.6–22.8% of rodents can carry *A. phagocytophilum* bacteria, similar to those (5.26% for *gltA* amplification and 31.57% for 16S rRNA PCR and DNA sequencing) described in our study.

Molecular typing based on single nucleotide polymorphisms (SNPs) in genes is the most frequently used method to analyze *A. phagocytophilum* genetic diversity. The most intensively used markers are the *groESL* operon, 16S rRNA locus, and the *ankA* and *msp2* genes. One previous study of species-specific 16S rRNA in the USA isolated the human pathogenic variant AP-ha (EP-ha, *Ehrlichia phagocytophila*-human agent) from other non-pathogenic variants (16). In Europe, the use of the species-specific 16S rRNA also clearly discriminated variants infecting red deer from those infecting roe deer (17,18). However, the conclusions were challenged by other studies in which this marker showed poor resolution and did not confirm any host species segregation for *A. phagocytophilum* variants (19). Finally, species-specific 16S rRNA data is unable to distinguish variants according to their geographical origins (20). In the present study, in Anyi County, we used PCR amplification of genus-specific 16S rRNA fragments extracted from individuals of different rodent species and DNA sequencing to classify *A. phagocytophilum* into 3 variants. Despite the identical geographical origins of the microorganisms in the same host species, they were dispersedly distributed in the phylogenetic tree.

The *gltA* gene encodes citrate synthase which catalyzes the condensation of acetyl coenzyme A with oxaloacetate to form citrate and has been assumed to be an important control point for determining the metabolic rate of the cell. The *A. phagocytophilum gltA* gene has multiple gene polymorphisms. *A. phagocytophilum* isolated from different dog samples showed diverse SNPs in the *gltA* gene and could be sub-grouped by alpha and beta variants (21). Analysis of the *gltA* sequences shows that our strain, in combination with that of a rodent from south-eastern China, was unique compared with all other variants, branching out from the group of sequences isolated from dog and sheep hosts in Spain, and tick vectors in the USA and Spain. Our study indicated that polymorphisms in the *A. phagocytophilum gltA* gene are probably related to the hosts and their geographical distribution.

Taken together, our results suggested that commensal rodents are the reservoirs for *A. phagocytophilum*, and controlling the rodent population wound make a great contribution to preventing HGA in Nanchang, China.

Acknowledgments The authors wish to acknowledge Yuanping Deng (the director of Anyi Center for Disease Control and Prevention) and Jianping He (the director of Xinjian Center for Disease Control and Prevention) for technical assistance during the collection of samples. This work was supported by Jiangxi Provincial Department of Science and Technology (2016BBG70005) and Health and Family Planning Commission of Jiangxi Province (20162007). Funders had no role in study design, the collection, analysis and interpretation of data, the writing of the report, and the decision to submit the article for publication. Weiqing Zheng was partially supported by Sasakawa Medical Fellowship through Japan-China Medical Association.

Conflict of interest None to declare.

REFERENCES

1. Lerdthusnee K, Nigro J, Monkanna T, et al. Surveys of rodent-borne disease in Thailand with a focus on scrub typhus assessment. Integr Zool. 2008;3:267-73.
2. Meerburg BG, Singleton GR, Kijlstra A. Rodent-borne diseases and their risks for public health. Crit Rev Microbiol. 2009;35:221-70.
3. De la Fuente J, Estrada-Peña A, Cabezas-Cruz A, et al. *Anaplasma phagocytophilum* uses common strategies for infection of ticks and vertebrate hosts. Trends Microbiol. 2016;24:173-80.
4. Dumler JS, Choi KS, Garcia-Garcia JC, et al. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. Emerg Infect Dis. 2005;11:1828-34.
5. Centers for Disease Control and Prevention (CDC). Annual cases of anaplasmosis in the United States. 2016; Available at <http://www.cdc.gov/anaplasmosis/stats/index.html>. Accessed June 25, 2017.
6. Fang LQ, Liu K, Li XL, et al. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis. 2015;15:1467-79.
7. Chastagner A, Moinet M, Perez G, et al. Prevalence of *Anaplasma phagocytophilum* in small rodents in France. Ticks Tick Borne Dis. 2016;7:988-91.
8. Hartwig V, von Loewenich FD, Schulze C, et al. Detection of *Anaplasma phagocytophilum* in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) from Brandenburg, Germany. Ticks Tick Borne Dis. 2014;5:277-80.
9. Yang J, Liu Z, Guan G, et al. Prevalence of *Anaplasma phagocytophilum* in ruminants, rodents and ticks in Gansu, northwestern China. J Med Microbiol. 2013;62:254-8.
10. Zhang L, Wang G, Liu Q, et al. Molecular analysis of *Anaplasma phagocytophilum* isolated from patients with febrile diseases of unknown etiology in China. PLoS One. 2013;8:e57155.
11. Kauffmann M, Rehbein S, Hamel D, et al. *Anaplasma phagocytophilum* and *Babesia* spp. in roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*) and mouflon (*Ovis musimon*) in Germany. Mol Cell Probes. 2017;31:46-54.
12. Santos HA, Thomé SM, Baldani CD, et al. Molecular epidemiology of the emerging zoonosis agent *Anaplasma phagocytophilum* (Foggie, 1949) in dogs and ixodid ticks in Brazil. Parasit Vectors. 2013;6:348.
13. Zheng W, Liu X, Guo X, et al. Study of community structure and seasonal fluctuation of rats in Nanchang. Chin J Vector Bio Control. 2011;22:446-8. Chinese.
14. Simuunza M, Weir W, Courcier E, et al. Epidemiological analysis of tick-borne diseases in Zambia. Vet Parasitol. 2011;175:331-42.
15. Cao W, Zhan L, He J, et al. Natural *Anaplasma phagocytophilum* infection of ticks and rodents from a forest area of Jilin Province, China. Am J Trop Med Hyg. 2006;75:664-8.
16. Massung RF, Mauel MJ, Owens JH, et al. Genetic variants of *Ehrlichia phagocytophila*, Rhode Island and Connecticut. Emerg Infect. Dis. 2002;8:467-72.
17. Petrovec M, Bidovec A, Sumner JW, et al. Infection with *Anaplasma phagocytophila* in cervids from Slovenia: evidence of two genotypic lineages. Wien Klin Wochenschr. 2002;114:641-7.
18. Zeman P, Pecha M. Segregation of genetic variants of *Anaplasma phagocytophilum* circulating among wild ruminants within a Bohemian forest (Czech Republic). Int J Med Microbiol. 2008;298:203-10.
19. Dugat T, Lagree AC, Maillard R, et al. Opening the black box of *Anaplasma phagocytophilum* diversity: current situation and future perspectives. Front Cell Infect Microbiol. 2015;5:61.
20. Casey ANJ, Birtles RJ, Radford AD, et al. Groupings of highly similar major surface protein (p44)-encoding paralogues: a potential index of genetic diversity amongst isolates of *Anaplasma phagocytophilum*. Microbiology. 2004;150:727-34.
21. Shukla SK, Aswani V, Stockwell PJ, et al. Contribution of polymorphisms in *ankA*, *gltA*, and *groESL* in defining genetic variants of *Anaplasma phagocytophilum*. J Clin Microbiol. 2007;45:2312-5.

ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol Vol. 56, No. 6: 589-596, December 2018
https://doi.org/10.3347/kjp.2018.56.6.589

▣ ORIGINAL ARTICLE

Tick-Borne Pathogens in Ixodid Ticks from Poyang Lake Region, Southeastern China

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Abstract: Ticks are the vectors of various pathogens, threatening human health and animal production across the globe. Here, for the first time we detected *Ricketssia* spp., *Borrelia* spp. and protozoan in ticks from Poyang Lake region in Jiangxi Province of eastern China. In 3 habitat categories and on 12 host species, 311 ticks from 11 species were collected. *Haemaphysalis longicornis* was the predominant species, accounting for 55.63%, followed by *Rhipicephalus microplus, Haemaphysalis flava* and *Ixodes granulatus*. Of the collected ticks, 7.07% were positive for tick-borne pathogens, and *H. longicornis* and *H. flava* were found to be co-infected with *Ricketssia* spp. and protozoan. *H. flava* was the most detected positive for tick-borne pathogens, whereas *H. longicornis* had the lowest infection rate, and the difference in infection rates between tick species was significant ($\chi^2=61.24$, $P<0.001$). Furthermore, adult ticks demonstrated remarkably greater infection rate than immature ticks ($\chi^2=10.12$, $P=0.018$), meanwhile ticks on Erinaceidae showed significantly higher positivity than ticks collected on other host species ($\chi^2=108.44$, $P<0.001$). Genetic fragment sequencing and analyses showed at least 4 pathogen species presence in ticks, namely *Borrelia yangtzensis, Rickettsia slovaca* or *Rickettsia raoultii* related genospecies, *Babesia vogeli* and *Hepatozoon canis* or *Hepatozoon felis* related genospecies. The finding indicates that the abundant ticks can carry diverse pathogens in Poyang Lake region, and pathogen infection is highly related to species, vertebrate hosts and life stages of ticks.

Key words: Tick-borne pathogens (TBPs), tick, epidemiology, risk factors, Poyang Lake region

INTRODUCTIOIN

Ticks, a group of specialized obligate hemophagous ectoparasites, parasitize abundant host species and are the vectors of wide range of pathogens of veterinary and public health importance [1-6]. Recently, they are considered to occupy the second place after mosquitoes as vectors of human infectious diseases in the world. As of May 31 2015, there were at least 5,568 cases of human tick-borne diseases reported around China, including large number of patients with Lyme diseases and newly emerging severe fever with thrombocytopenia syndrome [1].

China has the complex distributions and the great diversity of tick species because of its diverse ecological habitats. Ticks in China were reported to be carriers of various human pathogens including protozoans and bacterium like *Borrelia* spp. and *Richettsia* spp. [1,7,8]. Poyang Lake region, belonging to Jiangxi (a province of southeastern China), has already recorded sporadic human tick-borne diseases and at least 13 tick species. Our previous work detected some tick-borne pathogens in a few kinds of hosts, such as rodents and dogs in Poyang Lake region [4-6]. However, knowledge on tick-borne pathogens in tick vectors in this region is limited. Therefore, in this study we showed evidence to illustrate the distribution of pathogens comprising *Borrelia* spp., *Rickettsia* spp., and protozoa in tick vectors from Poyang Lake region in Jiangxi, and elucidated its relation with tick species, developmental stage, host and vegetation. The results will be a basis for future epidemiological studies and risk assessment of human tick-borne pathogens in Poyang Lake region.

• Received 30 August 2018, revised 9 October 2018, accepted 27 October 2018.
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MATERIAL AND METHODS

Study area

The study had been conducted for 3 years (2013-2015) in Poyang Lake region of Jiangxi Province, southeastern China, which has altitudes higher than 35 m and lower than 190 m above sea level. This area experiences a subtropical climate with over than 1,000 mm of annual rainfall, -10˚C of maximum low temperature and 40˚C of maximum high temperature. Temperatures usually vary from 10 to 37˚C between May and October when tick populations are active. Types of vegetation cover include mixed broadleaf and coniferous woodland and grassland (Table 1). We selected 12 counties in Poyang lake region as investigation sites (Table 1).

Tick collection and identification

Ticks in vegetation covers were collected by flagging or dragging both at ground level and over and through the vegetation with a cotton cloth (100×60 cm). Each site was visited at least 3 times to cover all of 3 categories of habitats (grassland, woodland, and shrubs). Each habitat category was selected to cover a 900-m^2 area with many animal trails and tracks. Ticks were removed from the cotton cloth every 2 minutes. Ticks parasitizing hosts were collected from 24 villages and 12 wild animal markets. In villages, domestic animals and fowls were restricted by owners for sampling. In markets, wild animal bodies were employed for tick collection. Rodentia around villages were trapped using peanut baited rodent traps for tick examination. All the procedures were carried out according to ethical guidelines for the use of animal samples permitted by Obihiro University of Agriculture and Veterinary Medicine (Animal experiment access num: 28-100). The information regarding all of the collected specimens, including their location, vegetation type, host, number of ticks collected from the body of each animal and the date of collection, were recorded. Ticks were collected from the entire body of each host into separate sample bottle containing 70% ethanol. Standard taxonomic keys were used to morphologically identify adults [9]. Larvae and nymphs were identified individually based on molecular methods [10]. The specimens were kept in 70% ethanol and used for further molecular identification and detection of tick-borne pathogens.

Table 1. Location and vegetation type of 12 plots sampled in this study

Location	Geographic coordinates	Vegetation type	Year surveyed
Anyi	N 28.6173°, W 115.5423°	G, S, W	2014, 2015
Wanli	N 28.8400°, W 115.7589°	W	2014
Xinjian	N 28.9800°, W 115.9154°	G	2014
Qingyunpu	N 28.6389°, W 115.9127°	G	2013, 2014
Duchang	N 29.2542°, W 116.1946°	W	2015
Hukou	N 29.7469°, W 116.2330°	W	2015
Wuning	N 29.2574°, W 115.0986°	G, S	2015
Poyang	N 29.0000°, W 116.6730°	G	2015
Wannian	N 28.6899°, W 116.9728°	W	2015
Wuyuan	N 29.2709°, W 117.75793°	G, S, W	2015
Yichun city	N 27.5914°, W 114.3252°	G, S, W	2015
Xingan	N 27.7327°, W 115.3791°	G, S, W	2015

W, woodland; S, shrubs; G, grassland.

DNA isolation

Tick specimens immersed in 70% ethanol were air dried, and then rinsed in sterile water for 3 times. After rinsed in sterile phosphate-buffered saline, ticks were dried on sterile filter paper in a biosafety hood, and individually ground in sterile tubes. DNA was extracted using the QIAamp Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The genomic DNA was stored at 4˚C until used as a template in PCR assays.

Pathogen identification

A total of 3 groups of pathogens were assayed: *Borrelia* spp., *Rickettsia* spp. and protozoa. A conventional PCR was performed with a set of primers (forward: 5′-ACATATTCAGATGCAGACAGAGGT-3′, reverse: 5′-GCAATCATAGCCATTGCAGATTGT-3′) designed to amplify the 665-bp flagellin gene of *Borrelia* spp. For citrate synthase encoding gene (*glt*A), a primer set of primer 1 (5′-GCAAGTATCGGTGAGGATGTAAT-3′) and primer 2 (5′-GCTTCCTTAAAATTCAATAAATCAGGAT-3′) was used and expected to yield a 401-bp fragment depending on the *Rickettsia* spp. For amplification of 209-214 bp fragment of 18S ribosomal RNA (rRNA) in the protozoa, a set of primers (forward: 5′-GCATTTAGCGATGGACCATTCAAG-3′, reverse: 5′-CCTGTATTGTTATTTCTTGTCACTACCTC-3′) was designed for PCR. PCR reagents were used as recommended by the manufacturer (Takara Bio Inc., Dalian, China). The amplification for *flagellin* gene included 5 min pre-denaturation at 94˚C followed by 30 cycles of denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 1 min, and final extension at 72˚C for 7 min. The amplifications for 18S rRNA gene in the protozoa and *glt*A gene in the *Rickettsia* spp. were performed under the

same conditions as *flagellin* gene except the extension at 72˚C for 45 sec for the protozoa and the annealing at 50˚C for 30 sec. Positive samples were sequenced to identify potential microbial species with a resemblance to known species based on by an online software (http://www.bioinformatics.org/sms2/ident_sim.html).

Sequence analysis

All obtained sequences were assembled and edited by using SeqMan software. We compared them with sequences in the GenBank database. We performed multiple sequence alignments by using the ClustalX program. Phylogenetic trees were constructed by using the Neighbor-Joining (NJ) algorithm in the MEGA v.7.0.26 software. Support for the tree nodes was calculated with 1,000 bootstrap replicates.

Data analyses

All the raw data were collated in Excel spreadsheets. The differences in infection rates of ticks at species levels, at developmental stages, on hosts, in habitat categories, and the difference in infection rates of ticks collected in vegetation covers and on hosts were evaluated using Chi square (http://quantpsy.org). In the 2×2 case of the chi-square test of independence, if expected frequencies is less than 5, Yates' correction is employed [11].

RESULTS

Tick samples

A total of 311 ticks belonging to 5 genera and 11 species were collected from 5 species of domestic animals (*Canis familiaris, Capra aegagrus hircus, Bos* spp., *Bubalus bubalis*, and *Equus ferus*), 5 species of wild animals (*Lepus sinensis*, Erinaceidae, *Apodemus agrarius, Rattus norvegicus, Rattus rattoides*), a species of bird (*Phasianus colchicus*) and a species of chicken (*Gallus gallus domesticus*), in 2 kinds of vegetation types from 12 locations in Poyang Lake region (Table 1). *L. sinensis* harbored abundant

Table 2. Summary of species and number of ticks collected from hosts and by flagging over vegetation cover

Vegetation covers/Animal hosts	Tick species	No. of ticks collected			Density[a]
		L	N	A	
Woodland (a=800 m^2)	*Haemaphysalis longicornis*	0	0	2M	0.0038
	Dermacentor auratus	0	0	1M	
Grassland (a=800 m^2)	*H. longicornis*	3	34	5M6F	0.06
Subtotal (a=1,600 m^2)	-	3	34	8M6F	0.032
Canis familiaris (n=24)	*H. longicornis*	0	5	4M25F	1.79
	R. sanguineus	0	0	1M8F	
Capra aegagrus hircus (n=44)	*H. longicornis*	0	2	0	0.80
	Haemaphysalis flava	2	2	3M1F	
	Rhipicephalus microplus	0	8	6M11F	
Bos spp. (n=13)	*H. longicornis*	1	8	0	4.31
	R. microplus	0	20	8M19F	
Bubalus bubalis (n=7)	*H. longicornis*	0	0	1F	1
	R. microplus	0	0	5F	
	Amblyomma testudinarium	0	0	1F	
Phasianus colchicus (n=5)	*Haemaphysalis phasiana*	0	5	0	1
Lepus sinensis (n=22)	*H. longicornis*	57	12	5M1F	3.77
	Ixodes acuminatus	0	0	1F	
	Ixodes sinensis	0	0	2M2F	
	Rhipicephalus haemaphysaloides	3	0	0	
Erinaceidae (n=3)	*H. flava*	0	1	3M9F	4.33
Apodemus agrarius (n=206)	*Ixodes granulatus*	0	0	1M4F	0.02
Rattus norvegicus (n=95)	*I. granulatus*	0	0	3M1F	0.04
Rattus rattoides (n=8)	*I. granulatus*	0	5	2M	0.88
Equus ferus (n=6)	*H. longicornis*	0	0	1F	0.17
Gallus gallus domesticus (n=30)	*H. longicornis*	1	0	0	0.03
Subtotal (n=463)	-	64	68	38M90F	0.56
Total (n=463; a=1,600 m^2)	-	67	102	46M96F	-

L, larvae; N, nymph; A, adult; M, male; F, female.
[a]Tick population density is denoted as ticks/hosts for ticks on hosts, ticks/m^2 for ticks collected from vegetation covers.

ticks such as *Haemaphysalis longicornis, Ixodes acuminatus, Ixodes sinensis* and *Rhipicephalus haemaphysaloides*, with the third highest tick population density of 3.77 ticks per a host. Hosts with the first highest and second highest tick loads were Erinaceidae (4.33 ticks per host) and *Bos* spp. (4.31 ticks per host), respectively. Other hosts with higher tick abundance were *B. bubalis* and *C. aegagrus hircus*, harboring 3 tick species. Sixty-seven female (14.79%) and 102 male (30.87%) adult ticks were obtained. Sixty-seven larvae and 102 nymphs accounted for 21.54% and 32.80% of the total number of ticks collected respectively. Of the 11 tick species collected, 3 species belonged to the genus *Haemaphysalis*, 3 species belonged to the genus *Rhipicephalus*, 3 species belonged to *Ixodes*, 1 species belonged to *Dermacentor*, and 1 other species belonged to the genus *Amblyomma*. The most abundant species was *H. longicornis* (55.63%), found in a kind of vegetation cover and infesting the most diverse host species (7 species). Three other common species included *H. flava*, *R. microplus* and *I. granulatus* (Tables 2, 3).

Pathogen infections in ticks

Protozoa, *Borrelia* spp. and *Rickettsia* spp. were detected in 4 tick species. Overall, 7.07% of ticks were tested positive for at least 1 pathogen. In detail, 2.31% of *H. longicornis* were detected positive for *Rickettsia* spp., or/and Protozoa, 18.75% of *I. granulatus* for *Borrelia* spp., 52.38% of *H. flava* for protozoa or/and *Rickettsia* spp. and 5.19% of *R. microplus* for protozoa. Infection rate in *H. flava* was significantly greater than that in *H. longicornis* ($\chi^2 = 61.24$, $P < 0.001$). Coinfection with protozoa and *Rickettsia* were found in *H. longicornis* and *H. flava*, with coinfection rate of 0.58% and 47.62%, respectively. There was no positive samples found in 7 tick species (*H. phasiana, I. acuminatus, R. sanguineus, R. haemaphysaloides, I. sinensis, A. testudinarium* and *D. auratus*) (Table 3).

The effect of risk factors on the pathogen distribution

The overall prevalence of pathogens in larvae, nymphs, male, and female ticks were 1.49%, 2.94%, 10.87%, and 13.54%, respectively. There was major difference in the prevalence of these pathogens between immatures (larvae and nymphs) and matures (males and females) ($\chi^2 = 10.12$, $P = 0.018$). However, there was no significant difference in prevalence of these pathogens in ticks among host species and vegetation, although the positive rate of pathogens in ticks collected from hosts was approximately 2 times more than that collected by flagging over vegetation ($\chi^2 = 0.44$, $P = 0.51$). Prevalence of these pathogens in ticks collected from *Canis familiaris, C. aegagrus hircus*, Muridae and Erinaceidae were 4.65%, 11.43%, 18.75%, and 84.62%, respectively, and ticks on Erinaceidae were at significantly higher risk for pathogen infection compared to ticks on other hosts ($\chi^2 = 108.44$, $P < 0.001$). There was no positive ticks found on other host species. Prevalence of these pathogens in ticks in grasslands and woodland were 2.08% and 0, respectively, and there was on significant difference ($\chi^2 = 1.37$, $P = 0.24$) (Table 4).

Pathogen identification and sequence analyses

Further sequencing and sequence alignment showed that 1 *Borrelia* species (*Borrelia yangtzensis*), 2 protozoan species (*Babesia vogeli* and *Hepatozoon canis* or *Hepatozoon felis* related geospecies), and 1 *Rickettsia* species (*Rickettsia slovaca* or *Rickettsia raoultii* related genospecies) were successfully sequenced from 4 tick species. The 665-base pair sequence of *Borrelia* spp. flagellin gene (MG717513) yielded in the study was 99.21-99.37% identical to other 2 sequences of MG717514 and MG717515 pro-

Table 3. Pathogen infection rates in ticks collected in Poyang Lake region

Tick species (number collected)	*Borrelia*	*Rickettsia*	Protozoa	Protozoa+ *Rickettsia*	Infection rate (%)	χ^2	*P*-value
Haemaphysalis longicornis (n=173)	0	4 (2.31)	1 (0.58)	1 (0.58)	4 (2.31)	61.24	<0.001
Ixodes granulatus (n=16)	3 (18.75)	0	0	0	3 (18.75)		
Haemaphysalis flava (n=21)	0	11 (52.38)	10 (47.62)	10 (47.62)	11 (52.38)		
Rhipicephalus microplus (n=77)	0	0	4 (5.19)	0	4 (5.19)		
Haemaphysalis phasiana (n=5)	0	0	0	0			
Ixodes acuminatus (n=1)	0	0	0	0			
Rhipicephalus sanguineus (n=9)	0	0	0	0			
Rhipicephalus haemaphysaloides (n=3)	0	0	0	0			
Ixodes sinensis (n=4)	0	0	0	0			
Amblyomma testudinarium (n=1)	0	0	0	0			
Dermacentor auratus (n=1)	0	0	0	0			

duced in the study. When compared to other fragments deposited in GenBank, MG717513 showed 98.73-98.89% identity to *B. yangtzensis* (EU135599, EU135601, and EU135602), 98.57-98.73% identity to *Borrelia valaisiana* (AB022134 and AB022135), and 95.25% identity to *Borrelia burgdorferi* sensu lato (X75202, X63413, and D63364). Therefore, 3 individuals of *Borrelia* spp. in the study were identified as *B. yangtzensis* or *B. yangtzensis*-related species. In *Rickettsia* spp., the 401 base-pair sequence of *glt*A gene (MG717516) obtained in a *H. longicornis* tick collected in grassland was 100% identical to the sequences of *glt*A gene isolated from 2 *H. longicornis* ticks (MG717517 and MG717523) on *C. familiaris* and 10 *H. flava* ticks on Erinaceidae (MG717518-MG717522, MG717524-MG717528), and 96.26% identical to the sequence in a *H. flava* tick on Erinaceidae (MG717529) (Table 5; Fig. 1). Our 13 sequences (MG717516-MG717528) showed 99.75% identity to the sequences of *R. raoultii* (MF002517) and *R. slovaca* (MF002529) deposited in GenBank, in addition, 1 remaining sequence (MG717529) presented 96.01% identity to *R. raoultii* and *R. slovaca*. The *Rickettsia* spp. pathogens in the study were identified as *R. raoultii* or *R. slovaca* related genospecies. Of 15 protozoa-positive specimens for amplification of 209-214 base-pair 18S ribosomal RNA by means of PCR method, 2 specimens were successfully sequenced (Table 5). The closest matches of 209 base-pair 18S ribosomal RNA of protozoa in our study were *B. vogeli* isolated in dogs from Jiangsu, China (MG586235, 100%), Serbia (KY747491, 100%), and Argentina (KY290978, 99%), and in *R. sanguineus* from India (MG050159, 100%) and from Australia (MG758132, 100%), in *Haemaphysalis concinna* from Czech Republic (KX8 57477, 100%). The 214 base-pair 18S ribosomal RNA of protozoa (MG675579) isolat-

Table 4. Comparison of difference of collected ticks and positive rates of pathogens among ticks by life stage, host species and vegetation type

Group		Sampled ticks	Positive ticks		χ^2	*P*-value
			No.	%		
Life stage	Larvae	67	1	1.49	10.12	0.018
	Nymph	102	3	2.94		
	Male	46	5	10.87		
	Female	96	13	13.54		
Vegetation type	Grassland	48	2	4.17	1.37	0.24
	woodland	3	0	0.00		
Host	Muridae	16	3	18.75	108.44	<0.001
	Canis familiaris	43	2	4.65		
	Capra aegagrus hircus	35	4	11.43		
	Lepus sinensis	83	0	0.00		
	Erinaceidae	13	11	84.62		
	Bubalus bubalis	7	0	0.00		
	Bos spp.	56	0	0.00		
	Phasianus colchicus	5	0	0.00		
Vegetation vs host	Vegetation	51	2	3.92	0.44	0.51
	Host	260	20	7.69		

Table 5. Pathogens in ticks collected from different hosts in different locations

Pathogens		Ticks species (No. positive)	Host species	Sampling site	GenBank accession No.
Borrelia	*B. yangtzensis*	*I. granulatus* (1♂1♀)	*R. norvegicus*	Anyi	MG717514-MG717515
		I. granulatus (1N)	*R. rattoides*	Anyi	MG717513
Rickettsia	*R. raoultii* or *R. slovaca* related genospecies	*H. longicornis* (2♀)	*Canis familiaris*	Xinjian, Poyang	MG717517, MG717523
		H. longicornis (1♀)	Grassland	Qingyunpu	MG717516
		H. flava (1N3♂1♀)	Erinaceidae	Hukou	MG717518-MG717522
		H. flava (1♂5♀)	Erinaceidae	Hukou	MG717524- MG717529
	Rickettsia sp.	*H. longicornis* (1L)	Grassland	Qingyunpu	-
Protozoa	*Babesia vogeli*	*H. flava* (1♂)	Erinaceidae	Hukou	MG675580
	Babesia sp.	*H. flava* (9♀)	Erinaceidae	Hukou	-
		R. microplus (1N3♀)	*Capra aegagrus hircus*	Yichun	-
	Hepatozoon canis or *Hepatozoon felis* related genospecies	*H. longicornis* (1♀)	Grassland	Qingyunpu	MG675579

ed in *H. longicornis* from grassland in the study showed 94.86% to *H. canis* (MG917719 and MG209594) and *H. felis* (KU232308), 92.99% identity to *Hepatozoon ursi* (KU232308), hence we proposed the protozoan as *H. canis* or *H. felis* related genospecies. *R. slovaca* or *R. raoultii* related genospecies was most frequently identified (14 times, 3 from the tick *H. longicornis*, 11 times from the tick *H. flava*), followed by *B. yangtzensis* (triple from *I. granulatus*). The other 2 protozoan species were detected only once. Twenty one of the 33 detections of pathogens were on *H. flava* collected from Erinaceidae (Table 5).

For phylogenetic analyses, 3 sequences of *B. yangtzensis flagellin* gene obtained from *I. granulatus* belonged to the same cluster where they shared with the strain QLZSP, QSYSP, and QTMP2 of *B. yangtzensis* and strain CKA3a and CMN1b of *B. valaisiana* (Table 5; Fig. 2). The sequences of *R. raoultii* or *R. slovaca* related *Rickettsia* spp. (MG717516-MG7175129) were clustered with those of *R. raoultii* (MF002517) and *R. slovaca* (MF002529) (Fig. 2).

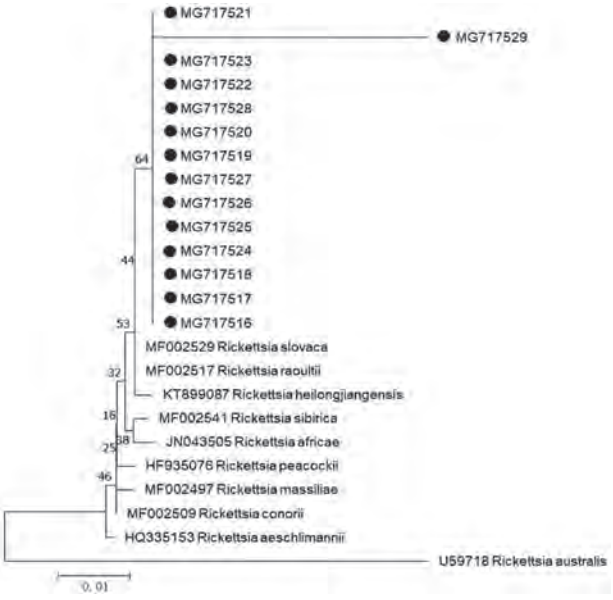

Fig. 1. Phylogenetic tree of *Rickettsia* spp. based on *glt*A gene. The trees were calculated by the neighbor-joining method using MEGA v.7.0.26 software. Values of the bootstrap support of the particular branching calculated for 1,000 replicated are indicated at the nodes. The variant sequences obtained from GenBank are designated by accession number and species. *Rickettsia australis* is used as outgroup. (●) denotes sequences of *R. slovaca* or *R. raoultii* related genospecies obtained in the study.

DISCUSSION

In Poyang Lake region, the common animals and birds with potential for tick parasitism and easy to contact human were *C. familiaris, C. aegagrus hircus, A. agrarius, R. norvegicus, G. gallus domesticus*, and *L. sinensis*, accounting for over 90% of hosts captured. Rodents like *A. agrarius* and *R. norvegicus* were trapped with large number, but a few ticks were found, whereas *B. yangtzensis* was occasionally detected in ticks removed from the rodents. *B. yangtzensis*, a *Borrelia* species in the *B. burg-*

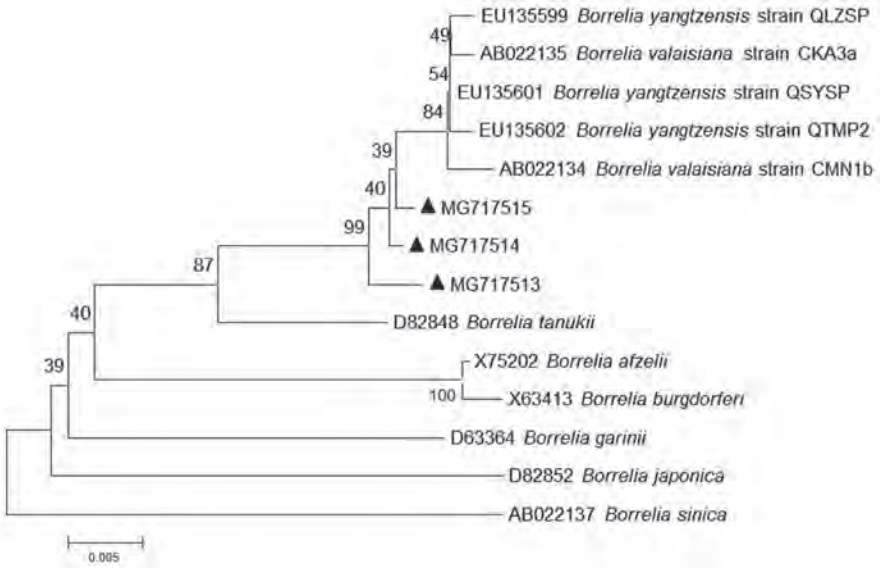

Fig. 2. Molecular phylogenetic tree of the *Borrelia* agent. The aligned nucleotide sequence of flagellin gene was subjected to analysis. Bootstrap 1,000 replicates are showed at the nodes. Scale bars indicate nucleotide substitutions per sites. *Borrelia sinica* is used as outgroups. (▲) prior to accession numbers are the sequences in the study.

dorferi complex was originally discovered in Chinese Yangtze River Valley region in 2015, and it was reported in *H. longicornis* and *I. granulatus* ticks from small mammals in China and isolated in rodents or shrews in Japan [12]. However, *B. yangtzensis* was not detected in *H. longicornis* albeit greater than 50% ticks collected in the study were *H. longicornis*. The reason, we guessed, might be that *H. longicornis* was not an efficient vectors of *B. yangtzensis*, hence the pathogen was rarely presented in the ticks. Poyang Lake region belongs to part of Yangtze River Valley region, and has similar distribution pattern of ticks and tick related small mammals to other parts of Yangtze River Valley region, therefore *B. yangtzensis* can also be found in *I. granulatus* collected in rodents in our study. The sequences of *flagellin* gene in *B. yangtzensis* in the study showed higher identity to *B. valaisiana* than to other known Lyme Borreliosis group spirochaete species, which was in agreement with the previous study [12].

Despite some *L. sinensis* were majorly tick infested, pathogens were not found in those ticks. We had 3 Erinaceidae hosts, and found diverse pathogens like *R. slovoca* or *R. raoultii*-like genospcies and *Babesia* spp. in attached ticks with high infection rate. Ticks on Erinaceidae might serve as vectors within Erinaceidae populations in this region, thus readily leading to high infection rate. This increases the chance that ticks transport pathogens from a natural hedgehog cycle to other hosts, including humans [13]. *C. familiaris*, usually functioning as a guard dog and a pet in investigated sites, were closely related to human, furthermore, some ticks on dogs in our study were positive for *R. slovoca* or *R. raoultii* related genospcies which is likely considered as human pathogen. Dogs, incidental hosts for the agent of spotted fever group, can become infected by a bite of ixodid ticks, and then transmit the pathogens to human [14]. Therefore, people should avoid contact with such dogs and ticks.

In this study, 11 tick species were collected, with *H. longicornis* acting as the predominant species, and other common ticks included *H. flava*, *R. microplus* and *I. granulatus*. These common tick species were also reported in other subtropical regions of China like Zhejiang and Hubei [15]. Our findings indicated that *H. flava* and *H. longicornis* were the ticks frequently detected positive for presence of *R. raoultii* or *R. slovoca* related genospecies. *R. raoultii* and *R. slovoca* were reported as human pathogenic agents [3,16,17]. Previous researches showed that *R. raoultii* had been reported in northern regions of China [3,7], and *R. slovaca* recorded in Europe and Xinjiang, China [7,16,17]. Although natural infection with tick-borne pathogens occurs [1], other tick species like *R. sanguineus, R. haemaphysaloides, I. sinensis*, and *A. testudinarium* were tested negative for *Borrelia* spp., *Rickettsia* spp. and protozoa infection in our study. The possible reason might be because of a few numbers of ticks collected and thus decreasing the probability of pathogen detection.

Compared to immature ticks, mature ticks tended to pathogen infection, furthermore, we found that females had comparable positivity rate with males. In contrast, a study conducted in Europe showed higher pathogen infection rate in immatures than matures [18]. Our study demonstrated that relatively high infection rate were determined in adult ticks collected from hedgehogs. The result suggests hedgehogs functioning as important pathogen reservoirs, and corresponds with a previous indication that several species of birds played a role as Lyme disease spirochetal reservoirs infective to ticks [18]. Therefore, in some cases, positivity rate is not depended by tick developmental stage but by which reservoir hosts that ticks attach to. For vegetation types, grassland sheltered more ticks than woodland and shrubs, and there were some ticks infected with *R. slovaca* or *R. raoultii* related genospecies in grassland. However, non-infected ticks were found in woodland, in contrast to more than 6 tick-borne pathogens infection in ticks from French suburban woodland [19]. Workers and visitors for travelling in the field should pay more attention to questing ticks in grassland in prevention of occurrence of tick-borne diseases. In addition, people in this region should keep a distance from hosts with tick infestation, especially the hosts with high risk for human tick-borne pathogens including hedgehogs, dogs and rodents.

ACKNOWLEDGMENTS

This study was supported financially by Jiangxi Provincial Department of Science and Technology (grant number 2016 BBG70005); Nanchang Science and Technology Bureau (Hong Scientific Research Program [2016] No. 96 Item 77); and the Health and Family Planning Commission of Jiangxi Province (grant number 20162007). The sponsors have no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. The authors thank Rongman Xu, Yi Sun, and Ze Chen for morphological identification of tick species, and also wish to acknowledge the contribution of Yuanping

Deng (the director of Anyi Center for Disease Control and Prevention), for technical assistance during the collection of samples. Weiqing Zheng was partially supported by Sasakawa Medical Fellowship through Japan-China Medical Association.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Fang LQ, Liu K, Li XL, Liang S, Yang Y, Yao HW, Sun RX, Sun Y, Chen WJ, Zuo SQ, Ma MJ, Li H, Jiang JF, Liu W, Yang XF, Gray GC, Krause PJ, Cao WC. Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis 2015; 15: 1467-1479.
2. Saito K, Ito T, Asashima N, Ohno M, Nagai R, Fujita H, Koizumi N, Takano A, Watanabe H, Kawabata H. *Borrelia valaisiana* infection in a Japanese man associated with traveling to foreign countries. Am J Trop Med Hyg 2007; 77: 1124-1127.
3. Li H, Zhang PH, Huang Y, Du J, Cui N, Yang ZD, Tang F, Fu FX, Li XM, Cui XM, Fan YD, Xing B, Li XK, Tong YG, Cao WC, Liu W. Isolation and identification of *Rickettsia raoultii* in human cases: a surveillance study in three medical centers in China. Clin Infect Dis 2018; 66: 1109-1115.
4. Zheng WQ, Chen HY, Liu MM, Adjou Moumouni PF, Efstratiou A, Liu ZB, Xuan XN. First evidence of *Mycoplasma haemocanis* in China. Trop Biomed 2017; 34: 983-990.
5. Zheng WQ, Liu Y, Tao H, Li Z, Xuan X, Liu X, Adjou Moumouni PF, Wu Y, Liu W, Chen H. First molecular evidence of *Anaplasma phagocytophilum* in rodent population of Nanchang, China. Jpn J Infect Dis 2018; 71: 129-133.
6. Zheng W, Liu M, Adjou Moumouni PF, Liu X, Efstratiou A, Liu Z, Liu Y, Tao H, Guo H, Wang G, Gao Y, Li Z, Ringo AE, Jirapattharasate C, Chen H, Xuan X. First molecular detection of tick-borne pathogens in dogs from Jiangxi, China. J Vet Med Sci 2017; 79: 248-254.
7. Han R, Yang J, Niu Q, Liu Z, Chen Z, Kan W, Hu G, Liu G, Luo J, Yin H. Molecular prevalence of spotted fever group rickettsiae in ticks from Qinghai Province, northwestern China. Infect Genet Evol 2018; 57: 1-7.
8. Takada N, Masuzawa T, Ishiguro F, Fujita H, Kudeken M, Mitani H, Fukunaga M, Tsuchiya K, Yano Y, Ma XH. Lyme disease *Borrelia* spp. in ticks and rodents from northwestern China. Appl Environ Microbiol 2001; 67: 5161-5165.
9. Teng GF. Chinese Economic Insect Archive, Ixodidae. Beijing, China. Science Press. 1978, pp 1-174.
10. Lv J, Wu S, Zhang Y, Chen Y, Feng C, Yuan X, Jia G, Deng J, Wang C, Wang Q, Mei L, Lin X. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). Parasit Vectors 2014; 7: 93.
11. Preacher KJ. Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software]; Available from: http://quantpsy.org/. 2001.
12. Margos G, Chu CY, Takano A, Jiang BG, Liu W, Kurtenbach K, Masuzawa T, Fingerle V, Cao WC, Kawabata H. *Borrelia yangtzensis* sp. nov., a rodent-associated species in Asia, is related to *Borrelia valaisiana*. Int J Syst Evol Microbiol 2015; 65: 3836-3840.
13. Skuballa J, Petney T, Pfäffle M, Taraschewski H. Molecular detection of *Anaplasma phagocytophilum* in the European hedgehog (*Erinaceus europaeus*) and its ticks. Vector Borne Zoonotic Dis 2010; 10: 1055-1057.
14. Nicholson WL, Allen KE, McQuiston JH, Breitschwerdt EB, Little SE. The increasing recognition of rickettsial pathogens in dogs and people. Trends Parasitol 2010; 26: 205-212.
15. Lu X, Lin XD, Wang JB, Qin XC, Tian JH, Guo WP, Fan FN, Shao R, Xu J, Zhang YZ. Molecular survey of hard ticks in endemic areas of tick-borne diseases in China. Ticks Tick Borne Dis 2013; 4: 288-296.
16. Gouriet F, Rolain JM, Raoult D. *Rickettsia slovaca* Infection, France. Emerg Infect Dis 2006; 12: 521-523.
17. de Sousa R, Pereira BI, Nazareth C, Cabral S, Ventura C, Crespo P, Marques N, da Cunha S. *Rickettsia slovaca* infection in humans, Portugal. Emerg Infect Dis 2013; 19: 1627-1629.
18. Olsén B, Jaenson TG, Bergström S. Prevalence of *Borrelia burgdorferi sensu lato*-infected ticks on migrating birds. Appl Environ Microbiol 1995; 61: 3082-3087.
19. Reis C, Cote M, Paul RE, Bonnet S. Questing ticks in suburban forest are infected by at least six tick-borne pathogens. Vector Borne Zoonotic Dis 2011; 11: 907-916.

Experimental and Applied Acarology (2019) 77:93–104
https://doi.org/10.1007/s10493-018-0324-1

Preliminary investigation of ixodid ticks in Jiangxi Province of Eastern China

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Received: 24 August 2018 / Accepted: 20 October 2018 / Published online: 12 December 2018

Abstract
In recent years, a large effort has been made for tick surveys for public health importance around China, especially after outbreaks of severe fever with thrombocytopenia syndrome (SFTS) occurred in humans in 2009. In this paper, the preliminary species composition and population distribution of ticks in Jiangxi Province of Eastern China is reported. Ticks were collected in three habitats (grassland, shrubs and woodland) and from nine host groups in 12 sampling sites throughout Jiangxi Province between 2011 and 2018. Six tick species including *Haemaphysalis longicornis, Rhipicephalus sanguineus* sensu lato, *Haemaphysalis yeni, Haemaphysalis kitaoka, Ixodes sinensis* and *Dermacentor auratus* were collected from the vegetation. *Haemaphysalis longicornis* was most abundant tick species, accounting for 90.6% of the total ticks. *Haemaphysalis yeni* and *H. kitaoka* were newly recorded tick species in Jiangxi Province. Tick presence was remarkably greater in grassland (89.4%) than in woodland (9.4%) and shrubs (1.2%), and nymphs (68.2%) and larvae (19.1%) were more frequently found than adult females (6.6%) and males (6.0%). On hosts, a total of 1513 ticks, from 13 species and four genera, were collected. These were *H. longicornis, Haemaphysalis campanulata, Haemaphysalis flava, Haemaphysalis phasiana, H. yeni, H. kitaoka, Haemaphysalis hystricis, R. sanguineus* (s.l.), *Rhipicephalus haemaphysaloides, Rhipicephalus microplus*, *Ixodes granulatus, I. sinensis* and *Amblyomma testudinarium. Amblyomma testudinarium* was a newly recorded tick species in Jiangxi Province. Based on this investigation, *H. longicornis* was the most frequently collected species (30.5%) and widely distributed tick species of the total collection ticks (in 11 sampling sites). *Haemaphysalis longicornis* had a broad host range and its presence (hosts with at least one tick) was significantly greater on *Lepus sinensis* (33.3%) than on *Canis familiaris* (2.3%) ($\chi^2 = 23.68$, $p = 0.0013$). In addition, the number of *H. longicornis* collected on *L. sinensis* (64.0%) was higher than on other host groups. Of all ticks collected on hosts, different developmental stages were obtained, which included 347 larvae (22.9%), 249 nymphs (16.5%), 404 adult males (26.7%) and 513 females (33.9%) and sex distribution was relatively uniform. These data indicate that a broad range of tick species is widely distributed throughout Jiangxi Province in Eastern China.

Keywords Species composition · Population distribution · Ixodid ticks · Jiangxi · Eastern China

Extended author information available on the last page of the article

Introduction

Ticks can transmit a great variety of pathogenic micro-organisms including protozoan, viral and bacterial agents (Zheng et al. 2017a, b, 2018; Wu et al. 2013). They are surpassed only by mosquitoes as arthropod vectors for transmitting human diseases and have gained enormous notoriety for affecting animal production in the world (Goodman et al. 2005). Most ticks falling into two families: the hard ticks (Ixodidae) and the soft ticks (Argasidae), are widely distributed in five continents around the globe. The world's hard ticks comprises 244 species in the genus *Ixodes*, 167 species in the genus *Haemaphysalis*, 132 species in the genus *Amblylomma*, and 148 species in the genera A*nomalohimalaya, Bothriocroton, Cosmiomma*, *Dermacentor, Hyalomma, Margaropus, Nosomma, Rhipicentor* and *Rhipicephalus* in the family Ixodidae (Guglielmone and Nava 2014).

Zoogeographically, China is divided into Palaearctic and Oriental Realm, and has abundant tick species which form approximately 1/8 of tick species worldwide. As of 2009, Chen et al. reported that hard tick fauna of this area involving 104 species in the following genera: *Amblyomma* (8 species), *Anomalohimalaya* (2 species), *Dermacentor* (12 species), *Haemaphysalis* (44 species), *Hyalomma* (6 species), *Ixodes* (24species) and *Rhipicephalus* (8 species) (Chen et al. 2010). To date, 15 hard tick species are recognized in Jiangxi Province (Xu et al. 2016, 2017; Zheng et al. 2011; Chen et al. 2010; Liu et al. 2013), which is less than one-third of tick species reported in Fujian Province bordered by Jiangxi (Chen et al. 2010). There appears to be incomplete investigation in some areas of Jiangxi Province. Thus a further investigation is needed in these areas. In the present study, we preliminarily investigated species composition and the distribution of hard ticks from wide range of hosts and three types of habitats in whole region of Jiangxi Province of Eastern China.

Methods and materials

Investigation site

We established 12 sampling sites in nine counties and three cities in Jiangxi Province, including Hukou, Wuning, Duchang, Jing'an, Nanchang, Wuyuan, Poyang, Wannian, Xingan, Yichun, Xinyu and An'yuan from 2011 to 2018 (Fig. 1). Jiangxi experiences a subtropical climate with over than 1000 mm of annual rainfall, − 5 °C of maximum low temperature and 38 °C of maximum high temperature. Types of vegetation cover include mixed broadleaf and coniferous woodland, shrubs and grassland. Woodland mainly involves the trees from the family of Pinaceae, Taxodiaceae Fagaceae, Theaceae, Lauraceae and Hamamelidaceae, and other trees such as *Sassafras tsumu* and *Acer* spp. are occasionally observed. The predominant shrub species are *Quercus fabri, Castanea sequinii, Adinandra millettii, Lindera aggergata, Vaccinium bracteatum, Actinidia chinensis, Dalbergia hupeana, Rhus chinensis*, and *Rhododendron simsii* in Shrubs. Grassland is dominated by *Eleusine indica, Conyza Canadensis, Cynodon dactylon, Setaria viridis, Avena fatua, Artemisia argyi, Imperata cylin drical, Humulus scandens, Erodium stephanianum* and *Galium aparine*.

Fig. 1 Map of Jiangxi Province. Stars indicate investigation sites in the study. Hukou county (HK), Wuning county (WUN), Duchang county (DC), Wuyuan county (WY), Jing'an county (JA), Poyang county (PY), Nanchang city (NC), Wannian county (WAN), Yichun city (YC), Xinyu city (XY), Xingan county (XG), Anyuan county (AY)

Tick collection

Tick investigations in vegetation covers and on hosts were both conducted 8 times (one time a year during 2011–2018) in Nanchang, 2 times (one time in the year 2015 and other time in the year 2018) in Yichun, once in the remaining places (the year 2015 or 2018).

Each investigation of ticks in vegetation covers involved three categories of habitats (grassland, woodland, and shrubs), and each habitat category was selected in a 900-m^2 area with many animal trails and tracks. Ticks were collected by flagging or dragging both at ground level and over and through the vegetation and were checked every 2 min; Ticks from hosts were collected from two villages and one wild animal market in each investigation. In villages, domestic animals and fowls were restricted by owners for sampling. In markets, wild animal bodies were employed for tick collection. Rodentia around villages were trapped using peanut baited rodent traps for tick examination. All the procedures were carried out according to ethical guidelines for the use of animal samples permitted by Obihiro University of Agriculture and Veterinary Medicine (Animal experiment access num: 28–100). The information regarding all of the collected specimens, including their location, vegetation type, host, number of ticks collected from the body of each animal and the date of collection, were recorded. Tick were collected from the entire body of each host into separate sample bottle containing 70% ethanol.

Tick count and species identification

After transferred to the laboratory, ticks immersed in 70% ethanol were counted and identified to species level by morphology according to taxonomic keys (Walker et al. 2003; Estrada-peña et al. 2004; Teng 1978). For damaged samples or immature ticks of different species which cannot be distinguished morphologically, polymerase chain reaction (PCR) amplification of 680 base-pair sequence of cytochrome oxidase subunit 1 (COI) gene and sequencing methods were used for molecular identification (Lv et al. 2014). Briefly, DNA was extracted with Dneasy® Blood and Tissue Kit (Qiagen, Germany) from a damaged tick, larvae or nymph. The partial fragment of the cytochrome c oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) with primers COI-F and COI-R. Fifty micro-liter DNA amplified with EX-Taq polymerase (Takara, Dalian, China) from all taxa was sent to Shanghai Sangon Biotech for sequencing from both 3′ and 5′ terminals of sequences. The sequences obtained from the sequencing company were discarded if spectrum of many nucleotide peaks were undistinguished and overlapped each other, or sequence length was <200 bp. Screened sequences were aligned with the sequences deposited in GenBank on the website of National Center for Biotechnology (NCBI). The sequences were identified according to taxonomic names assigned to reference sequence in GenBank. In the study, 18 sequences were obtained and deposited in GenBank under accession number MG721036-MG721053. The fragments MG721036-MG721044 were identified as *Haemaphysalis longicornis*, fragments MG721045, MG721046 and MG721051 as *Ixodes granulatus*, fragment MG721047 as *Dermacentor auratus*, fragments MG721048-MG721050, and MG721053 as *Rhipicephalus microplus*, and fragment MG721052 as *Haemaphysalis flava*.

Data analyses

All the raw data were collated in Excel spreadsheets. The differences in infestation rate of ticks on hosts and prevalence of species specific ticks on hosts were evaluated using χ^2 tests (http://quantpsy.org). In the 2·2 case of the χ^2 test of independence, if expected frequencies is less than 5, Yates' correction is employed (Preacher 2001).

Results

During the year 2011 and 2018, a total of 680 ticks including 616 *H. longicornis* (90.6%), 46 *Rhipicephalus sanguineus* sensu lato (6.8%), 15 *Haemaphysalis yeni* (2.2%), one *Haemaphysalis kitaoka* (0.1%), one *Ixodes sinensis* (0.1%) and one *D. auratus* (0.1%) were collected from three habitat categories. Of tick species of the total collection ticks in habitats, *H. yeni* and *H. kitaoka* were newly recorded tick species in Jiangxi Province and the specimens were deposited in Medical Insect Museum of Beijing Institute of Microbiology and Epidemiology, Beijing, China. Tick presence was remarkably greater in grassland (89.4%) than in woodland (9.4%) and shrubs (1.2%). Nymphs (68.2%) and larvae (19.1%) had greater numbers than females (6.6%) and males (6.0%) in three habitat categories, and intensity in grassland (nymphs, 68.2%; larvae, 16.9%) was higher than in woodland (nymphs, 0; larvae, 2.2%) and shrubs (nymphs, 0; larvae, 0) (Table 1).

In this study, 2151 individuals of hosts involving 9 groups-Rodentia, *Canis familiaris, Phasianus colchicus*, Erinaceidae, *Lepus sinensis, Bubalus bubalis, Bos* spp., *Capra*

Table 1 Ticks in vegetation types

Habit categories	*R. sanguineus*	*H. longicornis*	*H. yeni*	*H. kitaokai*	*I. sinensis*	*D. auratus*
Grassland	0/0/0/2[a]	464/115/16/10			0/0/0/1	
Woodland	0/0/20/20	0/2/3/2	0/13/2/0	0/0/0/1		0/0/1/0
Shrubs	0/0/0/4	0/0/3/1				
Total	0/0/20/26	464/117/22/13	0/13/2/0	0/0/0/1	0/0/0/1	0/0/1/0

[a]Numbers of ticks are recorded as Larvae/nymphs/males/females

aegagrus hircus and *Ovis aries* were used for tick examination (Table 2). A total of 1513 ticks, from 13 species and four genera, were collected on hosts from 12 sampling sites in Jiangxi. The 13 tick species included *H. longicornis, Haemaphysalis campanulata, H. flava, Haemaphysalis phasiana, H. yeni, H. kitaoka, Haemaphysalis hystricis, R. sanguineus* (s.l.), *Rhipicephalus haemaphysaloides, R. microplus, I. granulatus, I. sinensis* and *Amblyomma testudinarium*. Of tick species of the total ticks collected from hosts, *A. testudinarium* was newly recorded tick species in Jiangxi Province and the specimen was deposited in Medical Insect Museum of Beijing Institute of Microbiology and Epidemiology, Beijing, China. *H. longicornis* was the most frequently collected species (32%) (Fig. 2) and widely distributed tick species of the total collection ticks (in 11 sampling sites) (Table 3). *R. sanguineus* (s.l.) was the second abundant tick species (25%) (Fig. 2) and distributed in three sampling sites (Table 3). *R. microplus* was the second most distributed tick species (in four sampling sites) and the third most frequently observed (Fig. 2; Table 3).

The difference in tick infestation rate between host groups was found to be statistically significant ($p < 0.01$) which was higher in *L. sinensis* (66.7%) and *O. aries* (52.9%) and lower in Rodentia (0.7%). Regarding prevalence of tick species on different hosts, *H. longicornis* was present on *C. familiaris, P. colchicus*, Erinaceidae, *L. sinensis, Bos* spp., *B. bubalis, C. aegagrus hircus* and *O. aries*, and the presence (hosts with at least one tick) was significantly greater on *L. sinensis* (33.3%) than on *C. familiaris* (2.3%) ($\chi^2 = 23.68$, $p = 0.0013$). *Rhipicephalus sanguineus* s.l. presence was significantly higher on *O. aries* (29.4%) than on *C. aegagrus hircus* (1.2%) ($\chi^2 = 17.37$, $p = 0.0038$). *Haemaphysalis flava* was observed on Erinaceidae and *C. aegagrus hircus*, however it significantly preferred to infest Erinaceidae (28.6%) to *C. aegagrus hircus* (1.2%) ($\chi^2 = 7.93$, $p = 0.0049$). We also found that *R. haemaphysaloides* had more likelihood to attach to *L. sinensis* ($\chi^2 = 8.68$, $p = 0.034$), *R. microplus* to *Bos* spp. ($\chi^2 = 7.34$, $p = 0.025$) (Table 2). Both *P. colchicus* (13.9 ticks/individual) and *L. sinensis* (16.2 ticks/individual) were tick infested with higher burden compared with Rodentia (0.1 ticks/individual) and *B. bubalis* (0.4 ticks/individual) (Table 4).

Tick distribution at different developmental stages was roughly uniform, with larvae 22.9%, nymphs 16.5%, females 26.7% and males 33.9%. Of three most abundant tick species, *H. longicornis* larvae had an overwhelming number, accounting for 58.8%, and all of them were collected from *L. sinensis* hosts; Nymphs frequently infested *P. colchicus* and adults were found in greater number on *C. familiaris* and *Bos* spp. However, over than 95.0% *R. sanguineus* s.l. belonged to female and male ticks, most of which were captured on *C. familiaris* and *O. aries*, and *R. microplus* distribution at different developmental stages was relatively uniform (Table 5).

Table 2 Prevalence of tick species on different hosts (%)

Host		Hl	Hc	Hf	Hp	Hy	Hk	Hh	Rs	Rh	Rm	Ig	Is	At	Total
Rodentia (1781)	I	0	0	0	0	0	0	0	0	0	0	13		0	13
	P	0	0	0	0	0	0	0	0	0	0	0.7	0	0	0.7
C. familiaris (171)	I	4	2	0	0	0	0	0	7	2	0	0	0	0	13
	P	2.3	1.2	0	0	0	0	0	4.1	1.2	0	0	0	0	7.6
P. colchicus (7)	I	1	0	0	2	0	0	0	1	0	0	0	0	0	3
	P	14.3	0	0	28.6	0	0	0	14.3	0	0	0	0	0	42.9
Erinaceidae (7)	I	1	1	2	0	0	0	0	0	0	0	0	0	0	3
	P	14.3	14.3	28.6	0	0	0	0	0	0	0	0	0	0	42.9
L. sinensis (21)	I	7	0	0	0	0	0	0	2	3	0	0	2	0	14
	P	33.3	0	0	0	0	0	0	9.5	14.3	0	0	9.5	0	66.7
B. bubalis (14)	I	1	0	0	0	0	0	0	0	0	1		0	1	2
	P	7.1	0	0	0	0	0	0	0	0	7.1	0	0	7.1	14.3
Bos spp. (48)	I	7	0	0	0	0	0	2	3	1	8	1	1	0	20
	P	14.6	0	0	0	0	0	4.2	6.3	2.1	16.7	2.1	2.1	0	41.7
C. aegagrus hircus (85)	I	7	1	1	0	2	1	0	1	2	2	0	1	0	11
	P	8.2	1.2	1.2	0	2.4	1.2	0	1.2	2.4	2.4	0	1.2	0	12.9
O. aries (17)	I	2	0	0	0	2	0	0	5	0	0	0	0	0	9
	P	11.8	0	0	0	11.8	0	0	29.4	0	0	0	0	0	52.9
		$\chi^2=23.68^{※}$ $p=0.0013$	$\chi^2=1.52$ $p=0.47$	$\chi^2=7.93$ $p=0.0049$	/	$\chi^2=1.30$ $p=0.25$	/	/	$\chi^2=17.37$ $p=0.0038$	$\chi^2=8.68$ $p=0.034$	$\chi^2=7.34$ $p=0.025$	$\chi^2=0.05$ $p=0.82$	$\chi^2=2.00$ $p=0.37$	/	$\chi^2=563.91$ $p<0.01$

Numbers in the bracket are hosts examined; I, number of hosts with tick infestation; P, the percentage of hosts with tick infestation and the specific hosts detected; "/" in the table denotes that data is unavailable; ※, In the 2×2 case of the χ^2 test of independence, if expected frequencies is less than 5, Yates' correction is employed. Hl, *H. longicornis*; Hc, *Haemaphysalis campanulata*; Hf, *Haemaphysalis flava*; Hp, *Haemaphysalis phasiana*; Hy, *H. yeni*; Hk, *H. kitaoka*; Hh, *Haemaphysalis hystricis*; Rs, *R. sanguineus* (s.l.); Rh, *Rhipicephalus haemaphysaloides*; Rm, *Rhipicephalus microplus*; Ig, *Ixodes granulatus*; Is, *I. sinensis* and At, *Amblyomma testudinarium*

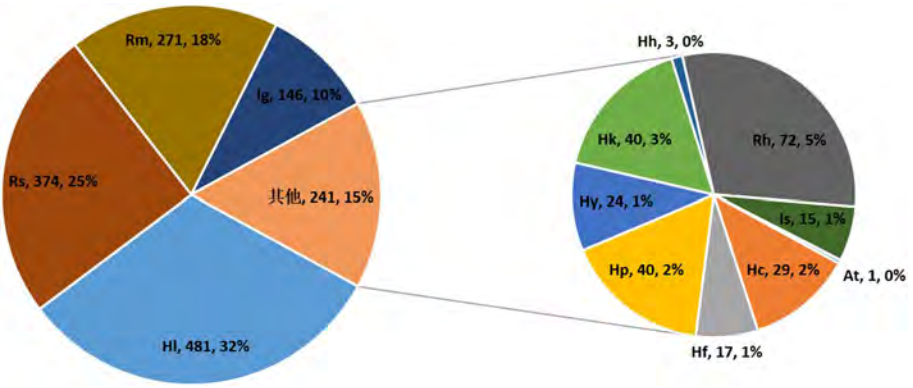

Fig. 2 Species composition of ticks infesting hosts. Hl, *H. longicornis*; Hc, *Haemaphysalis campanulata*; Hf, *Haemaphysalis flava*; Hp, *Haemaphysalis phasiana*; Hy, *H. yeni*; Hk, *H. kitaoka*; Hh, *Haemaphysalis hystricis*; Rs, *R. sanguineus* (s.l.); Rh, *Rhipicephalus haemaphysaloides*; Rm, *Rhipicephalus microplus*; Ig, *Ixodes granulatus*; Is, *I. sinensis* and At, *Amblyomma testudinarium*

Table 3 Geographical distribution of tick species in Jiangxi Province

Locality	Hl	Hc	Hf	Hp	Hy	Hk	Hh	Rs	Rh	Rm	Ig	Is	At	Da
HK			+	+				+						+
WUN	+												+	
DC	+													
WY	+													
JA	+				+	+								
PY	+													
NC	+	+	+	+	+			+	+	+	+	+		
WAN	+							+						
YC	+					+				+	+			
XY	+													
XG	+									+				
AY	+						+			+				
Jiangxi	+	⊞	+	+	⊞	⊞	+	+	+	+	+	+	⊞	+

+, denotes tick species were recognized in the locality. Cross with a box indicates newly recorded tick species in Jiangxi Province

Discussion and conclusion

Haemaphysalis longicornis is a kind of hard tick species, widely distributed in East Asia, Australia and New Zealand, and sometimes can be found in northernmost regions of Primorye (Northeastern USSR) and Hokkaido of Japan (Hoogstraal et al. 1968). In 2010, Chen et al. reported that *H. longicornis* was widely distributed tick species in various regions of China except for Jiangxi Province (Chen et al. 2010). In 2017, Xu et al. informed us of six newly reported tick species of Jiangxi, including *H. longicornis* (Xu et al. 2017). Information about *H. longicornis* presence in Jiangxi Province mirrors either previous incomplete

Table 4 Tick population densities on different hosts

Host	Hosts detected	Ticks on the hosts	Ticks per a host
Rodentia	1781	145	0.1
C. familiaris	171	341	2.0
P. colchicus	7	97	13.9
Erinaceidae	7	29	4.1
L. sinensis	21	341	16.2
B. bubalis	14	6	0.4
Bos spp	48	333	6.9
C. aegagrus hircus	85	129	1.5
O. aries	17	92	5.4
Total	2151	1513	0.7

tick investigation in the region or *H. longicornis* distribution expansion in China. Therefore, we performed the whole region tick investigation to obtain more data on tick species and their distribution in the region. Finally, we found that *H. longicornis*, the dominant species in the region, frequently collected in 11 sampling sites ranging from northern and southern part of Jiangxi Province, and found in three habitat categories and on *L. sinensis*, *P. colchicus*, *Bos* spp. and *C. familiaris* and, occasionally, on *O. aries, C. aegagrus hircus*, Erinaceidae and *B. bubalis*, which is in agreement with a former study conducted in some place of the region (Xu et al. 2017). Our findings are also consistent with what was previously reported in South Korea and Western Japan (Iwakami et al. 2014; Park et al. 2014). *Haemaphysalis longicornis* is easily observed in bush habitat and on cattle host. In our study, a large number of *H. longicornis* ticks were collected by flagging over a grassland of Nanchang, with some bushes growing in the grassland. In addition, we found that *H. longicornis* frequently infested Chinese hares not cattle in large number. Chinese hares, similar to the brown hare in New Zealand (Heath et al. 1987), range extensively and move long distance in open land including grassland, shrubs and forestland to facilitate *H. longicornis* dispersion. Cattle in sampling sites was grazed in good sanitary condition and regularly treated with some acaricides, which majorly reduced possibility of presence of *H. longicornis* on this host. However, above results were based on our primary study and need further verification under investigation of more numerous cattle and hares in the future.

Except *H. longicornis*, there was another abundant tick species *R. sanguineus* s.l. Previous data reported that *R. sanguineus* s.l. was confined to Nanchang, and infested *C. aegagrus hircus* and *L. sinensis* (Xu et al. 2017). Our study verified additional distribution of the species in Hukou and Wannian, its presence in woodland, grassland and shrubs, and more parasitizing *C. familiaris*, *P. colchicus*, *O. aries* and *Bos* spp. Canidae are hosts of all stages of *R. sanguineus* s.l. (Guglielmone et al. 2014), however, we only found female and male *R. sanguineus* s.l. on *C. familiaris*, albeit more than 250 ticks were collected. We mainly investigated tick infestation on *C. familiaris* in May–June in Jiangxi Province, and this term corresponded with mating time of the species in the region.

Previous studies indicate that adults (females and males) of some tick species feed mainly on medium- and large-sized animals, and the most important hosts for immature stages (larvae and nymphs) are small-sized mammals (Yu et al. 2011; Zheng et al. 2012). *L. sinensis*, *C. familiaris*, *P. colchicus*, *O. aries* and *Bos* spp. represent the main hosts for *H. longicornis* and *R. sanguineus* s.l. ticks in the current area investigated. Immatures failed to

Table 5 The compositions of ticks at different developmental stages on hosts

Tick genus	Tick species	Hosts	Larvae	Nymphs	Males	Females
Haemaphysalis	*longicornis*	*C. familiaris*	0 (0)[a]	0 (0)	25 (56.8)	19 (43.2)
		C. aegagrus hircus	0 (0)	10 (40)	5 (20)	10 (40)
		Bos spp.	0 (0)	0 (0)	22 (45.8)	26 (54.2)
		B. bubalis	0 (0)	0 (0)	0 (0)	1 (100)
		L. sinensis	283 (91.9)	13 (4.2)	3 (1.0)	9 (2.9)
		P. colchicus	0 (0)	40 (93.0)	3 (7.0)	0 (0)
		Erinaceidae	0 (0)	5 (100)	0 (0)	0 (0)
		O. aries	0 (0)	0 (0)	6 (85.7)	1 (14.3)
		Sub-total	283 (58.8)	68 (14.1)	64 (13.3)	66 (13.7)
	campanulata	*C. familiaris*	0 (0)	8 (38.1)	2 (9.5)	11 (52.4)
		C. aegagrus hircus	0 (0)	0 (0)	0 (0)	1 (100)
		Erinaceidae	0 (0)	0 (0)	4 (57.14)	3 (42.9)
		Sub-total	0 (0)	8 (27.6)	6 (20.7)	15 (51.7)
	flava	Erinaceidae	0 (0)	0 (0)	7 (41.2)	10 (58.8)
	phasiana	*P. colchicus*	0 (0)	0 (0)	13 (32.5)	27 (67.5)
	yeni	*C. aegagrus hircus*	0 (0)	4 (26.7)	6 (40)	5 (33.3)
		O. aries	0 (0)	0 (0)	0 (0)	9 (100)
		Sub-total	0 (0)	4 (16.7)	6 (25)	14 (58.3)
	kitaoka	Goat	0 (0)	40 (100)	0 (0)	0 (0)
	hystricis	*Bos* spp	0 (0)	0 (0)	2 (66.7)	1 (33.3)
Rhipicephalus	*sanguineus* s.l.	*C. familiaris*	0 (0)	0 (0)	129 (48.5)	137 (51.5)
		C. aegagrus hircus	0 (0)	0 (0)	0 (0)	1 (100)
		Bos spp.	0 (0)	0 (0)	4 (30.77)	9 (69.2)
		L. sinensis	0 (0)	1 (25)	3 (75)	0 (0)
		P. colchicus	14 (100)	0 (0)	0 (0)	0 (0)
		O. aries	0 (0)	0 (0)	48 (63.2)	28 (36.8)
		Sub-total	14 (3.7)	1 (0.3)	184 (49.2)	175 (46.8)
	haemaphysaloides	*C. familiaris*	0 (0)	0 (0)	8 (80)	2 (20)
		C. aegagrus hircus	0 (0)	0 (0)	1 (50)	1 (50)
		Bos spp.	0 (0)	0 (0)	13 (29.6)	31 (70. 5)
		L. sinensis	0 (0)	0 (0)	3 (18.8)	13 (81.3)
		Sub-total	0 (0)	0 (0)	25 (34.7)	47 (65.3)
	microplus	*C. aegagrus hircus*	15 (34.1)	16 (36.4)	7 (15.9)	6 (13.6)
		Bos spp.	24 (10.8)	67 (30.0)	44 (19.7)	88 (39.5)
		B. bubalis	0 (0)	4 (100)	0 (0)	0 (0)
		Sub-total	39 (14.4)	87 (32.1)	51 (18.8)	94 (34.7)
Ixodes	*granulatus*	*Bos* spp.	(0)	(0)	(0)	1 (100)
		Rodentia	11 (7.6)	41 (28.3)	42 (29.0)	51 (35.2)
		Sub-total	11 (7.5)	41 (28.1)	42 (28.8)	52 (35.6)

Table 5 (continued)

Tick genus	Tick species	Hosts	Larvae	Nymphs	Males	Females
	sinensis	*C. aegagrus hircus*	0 (0)	0 (0)	1 (100)	(0)
		Bos spp.	0 (0)	0 (0)	1 (100)	(0)
		L. sinensis	0 (0)	0 (0)	1 (7.69)	12 (92.3)
		Sub-total	0 (0)	0 (0)	3 (20)	12 (80)
Amblyomma	*testudinarium*	*B. bubalis*	0 (0)	0 (0)	1 (100)	0 (0)
Total			347 (22.9)	249 (16.5)	404 (26.7)	513 (33.9)

[a]In parentheses are the percentages of ticks at specific developmental stages

attack large-sized animal like *Bos* spp. Interestingly, overwhelming matures did not attach themselves to small-sized animal like *P. colchicus*. However, both matures and immatures can be simultaneously found on small- and medium-sized animals such as *Lepus sinensis. Haemaphysalis longicornis* and *R. sanguineus* s.l. follow a three-host life cycle. Immature ticks use these hosts only for feeding, while the adults may also apply hosts for seeking a mating partner. The different behavior might lead to more host-specific feeding, obviously adults for large-sized hosts and immatures for small-sized hosts. In addition, adult ticks have larger surface area to volume ratios and are therefore less sensitive to water stress, a major cause of mortality in smaller immature ticks. The difference in desiccation resistance is reflected by the position where adults and immatures quest for a host. Immatures usually stay somewhere near ground where vegetation covers create high humid environment and small-size animals are more available. Whereas, adults find potential hosts in higher vegetation layers, where they may miss small-sized host species (Esser et al. 2016).

Of the selected 12 sampling sites for tick investigation, there were 10 tick species found in Nanchang city, accounting for five-seventh of the total tick species detected. During 2011–2018, we performed the investigations annually in Nanchang, however, twice in Yichun and once in the remaining sampling sites because of lack of human, material and financial support. Lower tick species biodiversity may be explained by incomplete investigation in areas outside Nanchang city. Therefore, sampling effort should be increased for tick surveillance in other regions of Jiangxi Province to enrich the current data on tick species and their distribution in those regions.

Ticks can transmit various pathogen including viruses, protozoan parasites and bacteria. Yu et al. listed 51 important vector hard tick species in China in 2015, including 10 tick species found in this study, such as *I. granulatus, I. sinensis, A. testudinarium, H. longicornis, H. flava, H. kitaokai, H. yeni, D. auratus, R. microplus* and *R. sanguineus* s.l. *Haemaphysalis longicornis* is the vector of New Bunyavirus and *I. granulatus* can transmit *Borrelia burgdorferi*, both of which are greatly important pathogens to human health (Yu et al. 2015). Therefore, inhabitants in the region should reduce exposure to rodent hosts for *I. granulatus* in residential surrounding, and tourists and workers should keep away from hare hosts for *H. longicornis* in the field, in order to achieve the goal of preventing the bite of the two tick species and controlling severe fever with thrombocytopenia syndrome (SFTS) and Lyme disease, which were formerly reported in Jiangxi Province (Fang et al. 2015).

In conclusion, this study has preliminarily shown that like other regions in China, Jiangxi may have abundant tick populations. The results presented in this study highlights

tick species composition and their distribution in Jiangxi through an 8-year-long study. However, more tick investigations are needed in the areas outside Nanchang city for creating a full view of tick population distribution in Jiangxi. Further works regarding tick-borne pathogens detection are recommended in order to better understand the risk posed by the presence of tick populations in Jiangxi to human health and animal production.

Acknowledgements This study was supported financially by Jiangxi Provincial Department of Science and Technology (Grant Number 2016BBG70005); and funded by Health and Family Planning Commission of Jiangxi Province (Grant Number 20162007). The sponsors have no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. Meanwhile, the authors wish to acknowledge the contribution of Yuanping Deng (the director of Anyi Center for Disease Control and Prevention), for technical assistance during the collection of samples. Weiqing Zheng was partially supported by Sasakawa Medical Fellowship through Japan–China Medical Association.

References

Chen Z, Yang XJ, Bu FJ, Yang XH, Yang XL, Liu JZ (2010) Ticks (acari: ixodoidea: argasidae, ixodidae) of China. Exp Appl Acarol 51:393–404

Esser HJ, Foley JE, Bongers F, Herre EA, Miller MJ, Prins H, Jansen PA (2016) Host body size and the diversity of tick assemblages on Neotropical vertebrates. Int J Parasitol Parasit Wildlife 5:295–304

Estrada-peña A, Bouattour A, Camicas JL, Walker AR (2004) A guide to identification of species. European Union, Brussel

Fang LQ, Liu K, Li XL, Liang S, Yang Y, Yao HW, Sun RX, Sun Y, Chen WJ, Zuo SQ, Ma MJ, Li H, Jiang JF, Liu W, Yang XF, Gray GC, Krause PJ, Cao WC (2015) Emerging tick-borne infections in mainland China: an increasing public health threat. Lancet Infect Dis 15:1467–1479

Goodman JL, Dennis DT, Sonenshine DE (2005) Tick-Borne diseases of humans. ASM Press, Washington, DC, pp 363–392

Guglielmone AA, Nava SS (2014) Names for ixodidae (acari: ixodoidea): valid, synonyms, incertae sedis, nomina dubia, nomina nuda, lapsus, incorrect and suppressed names—with notes on confusions and names for ixodidae (acari: ixodoidea): valid, synonyms, incertae sedis, nomina dubia. Zootaxa 3767:1–256

Guglielmone AA, Richard GR, Apanaskevich DA, Trevor NP, Estrada-peña A, Horak IG (2014) The hard ticks of the world (Acari: Ixodida: Ixodidae). Springer-Verlag, Berlin, pp 303–586

Heath AG, Tenquist JD, Dallas MB (1987) Goats, hares, and rabbits as hosts for the New Zeal and cattle tick, *Haemaphysalis longicornis*. N Z J Zool 14:549–555

Hoogstraal H, Roberts HF, Kohls GM, Vernon JT (1968) Review of *haemaphysalis* (*kaiseriana*) *longicornis* neumann (resurrected) of Australia, New Zealand, New Caledonia, Fiji, Japan, Korea, and Northeastern China and ussr, and its parthenogenetic and bisexual populations (ixodoidea, ixodidae). J Parasitol 54:1197–1213

Iwakami S, Ichikawa Y, Inokuma H (2014) Molecular survey of *Babesia gibsoni* using *Haemaphysalis longicornis* collected from dogs and Cats, in Japan. J Vet Med Sci 76:1313–1316

Liu ZB, Ye JH, Li G (2013) Epidemiological survey of tick-borne diseases in police dogs. Chin Work Dog Ind 2:16–17

Lv JZ, Wu SQ, Zhang YN, Yan C, Feng CY, Yuan XF, Jia GL, Deng JH, Wang CX, Qin W, Lin M, Lin XM (2014) Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida)Ixodida. Parasit Vectors 7:93

Park S, Song BG, Shin EH, Seok-min Y, Myung-guk H, Park MY, Park C, Ryou U (2014) Prevalence of severe fever with thrombocytopenia syndrome virus in Haemaphysalis longicornis ticks in South Korea. Ticks Tick Borne Dis 5:975–977

Preacher KJ (2001) Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence. Calculation for the Chi-Square Test. Quantpsy.org. http://www.quantpsy.org/chisq/chisq.htm. Accessed 30 July 2018

Teng GF (1978) Chinese economic insect archive, ixodidae. Science press, Beijing, pp 1–174

Walker AR, Bouattour A, Camicas JL, Estrada-peña A, Horak IG, Latif AA, Pegram RG, Preston PM (2003) Ticks of domestic animals in Africa: a guide to identification of species. Bioscience Reports, Edinburgh, Scotland

Wu XB, Na RH, Wei SS, Zhu JS, Peng HJ (2013) Distribution of tick-borne diseases in China. Parasit Vectors 6:119
Xu HB, Xu ZJ, Huang H, Duan XL, Li YQ, Chen KF, Wang Q, Wu YY (2016) Two newly recorded species of Ixodidae in Jiangxi Province, China. Chin J Vect Biol Control 27:496–497
Xu HB, Huang H, Xu JZ, Tian JH, Wu DC, Wang Q (2017) Six newly recorded Ixodidae in Jiangxi Province, China. J Cent Chin Norm Univ Nat 51:804–808
Yu Z, Zheng H, Yang X, Chen Z, Wang D, Hao M, Yang Y, Liu J (2011) Seasonal abundance and activity of the tick *Dermacentor silvarum* in northern China. Med Vet Entomol 25:25–31
Yu Z, Wang H, Wang T, Sun W, Yang X, Liu J (2015) Tick-borne pathogens and the vector potential of ticks in China. Parasit Vect 8:24
Zheng WQ, Chen HY, Liu XQ, Xuejian G, Fu RL (2011) Severe tick infestation in a HARE and potential risk for transmitting pathogens to humans. Kor J Parasitol 49:419–422
Zheng HY, Yu ZJ, Zhou LF, Yang XL, Liu JZ (2012) Seasonal abundance and activity of the hard tick Haemaphysalis longicornis (Acari: Ixodidae) in North China. Exp Appl Acarol 56:133–141
Zheng WQ, Chen HY, Liu MM, Adjou MP, Efstratiou A, Liu ZB, Xuan XN (2017a) First evidence of Mycoplasma haemocanis in China. Trop Biomed 34:983–990
Zheng WQ, Liu MM, Moumouni PFA, Liu XQ, Efstratiou A, Liu ZB, Liu YQ, Tao HY, Guo HP, Wang GB, Yang G, Li ZF, Ringo AE, Jirapattharasate C, Chen HY, Xuan XN (2017b) First molecular detection of tick-borne pathogens in dogs from Jiangxi, China. J Vet Med Sci 79:248–254
Zheng WQ, Liu YQ, Tao HY, Li ZF, Xuan XN, Liu XQ,, Wu YY, Liu WQ, Chen HY (2018) Paul Franck Adjou moumouni. First molecular evidence of *Anaplasma phagocytophilum* in rodent population of Nanchang, China. Jpn J Infect Dis 80:129–133

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日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

Supported by 日本財団 THE NIPPON FOUNDATION

第40期　　研究者番号：G4002　　作成日：2019年3月10日

氏名	LIU JUE	劉 珏	性別	F	生年月日 1989.11.03
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研究先(指導教官)	筑波大学大学院　体育系　(大藏 倫博　准教授)				
研究テーマ	地域在住高齢者におけるフレイルと交通事故発生率およびリスクとの関連				
専攻種別	論文博士	□	課程博士	☑	

1．研究概要（1）

高齢化が急速に進む我が国において、高齢者の自動車運転中の交通事故の問題が社会的に大きくクローズアップされている。65歳以上の自動車運転免許保有者数が1,600万人を超え、高齢者の交通事故死者数は全死者数の54.7%を占めている（警察庁，2017）。高齢者の運転行動に影響を与える要因として、加齢による身体機能・認知機能・視覚機能の低下が指摘されている。これらの加齢に伴う機能変化などを、近年では『フレイル』という概念により定義することがある。フレイルを有する高齢者は、健常と要介護状態の中間の状態にあたり、転倒、入院、ADLや手段的ADLの障害、さらには要介護状態への移行だけでなく、交通事故の発生リスクも高いことが考えられる。これらのことから、高齢者のフレイル予防は、交通事故防止や安全な運転行動を適切に保つ上でも重要である。しかし、我が国の地域在住高齢者を対象として、フレイルと交通事故発生率との関連性、またフレイルな状態が将来の交通事故発生率およびリスクに及ぼす影響は定かではない。

1）目的（Goal）

本研究の目的は地域在住高齢者を対象として、フレイルと交通事故発生率との関連性を明らかとした上で、フレイルな状態が将来の交通事故発生率およびリスクに及ぼす影響について検討することである。

2）戦略（Approach）

上記の目的を達成するために、以下の課題を設定する。

・課題1　高齢運転者のフレイルと交通事故発生率との関連性

地域在住高齢者1万名を対象とした郵送調査により、フレイルと交通事故発生率との横断的関連を検討する。

・課題2　高齢運転者のフレイルな状態が将来の交通事故発生率およびリスクに及ぼす影響

課題2-1：高齢運転者におけるフレイルと身体・認知・視覚機能との関連

地域在住高齢者400名を対象として、フレイルを有する運転者と健常運転者の身体・認知・視覚機能の比較をおこなう。

課題2-2：高齢者におけるフレイルな状態が将来の交通事故発生率およびリスクに及ぼす影響（縦断的検討）

2018年時点でフレイルを有する高齢者が将来の交通事故発生率およびリスクに及ぼす影響を縦断的検討から明らかにする。

3）材料と方法（Materials and methods）

課題1では、対象者は茨城県笠間市在住高齢者1万人とし、郵送調査を実施する。基本チェックリストを用いてフレイルな状態を定義し、さらに運転行動（運転有無・免許取得年・運転頻度・時間）及び過去3年間の交通事故経験を詳細に調査する。

日本で独自に用いられているフレイルの指標として、厚生労働省が作成した基本チェックリストがよく使われている。基本チェックリストは生活状態や心身の機能に関する25の質問に対して、「はい」か「いいえ」で回答する自記式質問票である。

交通事故経験の定義は過去3年間に対象者ご自身が運転していて事故を起こした場合とし、(1)対人（人との接触）事故を起こした;(2) 対物（器物を損壊する）事故を起こした;(3)車をこすった、あるいはぶつけた;(4)車同士の事故を起こした。以上の4つの点から評価する。また、4つのうち、1つでもあてはまった場合、交通事故経験ありというふうに定義する。

3）材料と方法（Materials and methods）
課題2-1では、対象者はかさま長寿健診に参加する高齢者400名とし、測定や調査を実施する。測定会にて身体機能（Timed Up & Go Test(TUG)、5回椅子立ち上がり、開眼片足立ち、長座体前屈など）、認知機能(Trail Making Test A/B、ファイブ・コグおよび下位項目）および視機能（静止視力、動体視力）を評価する。また、Friedらによって報告されたCardiovascular Health Studyでの判定基準および基本チェックリストを用いてフレイルな状態を定義する。さらに、自動車の運転に関するアンケート（運転有無・運転頻度・免許取得年・運転時間・運転への期待・過去3年間の交通事故経験・過去3年間のヒヤリハット経験、補償運転有無など）を調査する。

課題2-2では、対象者は2018、2019、2020年かさま長寿健診に参加する高齢者300名とし、課題2-1同様に項目を評価する。

4）実験結果（Results）
＜期待される効果＞
高齢者が安全に運転を継続することは、自立した生活を送る上で重要な要因である。本研究により、近年注目されている『フレイル』という概念が将来の交通事故発生率およびリスクに及ぼす影響を明らかとなれば、フレイル予防や早期発見・介入が高齢者の交通事故防止という観点からも重要であることを示すことができる。

5）考察（Discussion）
6）参考文献（References）
1. Sewo Sampaio, P. Y., Sampaio, R. A., Coelho Junior, H. J., Teixeira, L. F., Tessutti, V. D., Uchida, M. C., & Arai, H. (2016). Differences in lifestyle, physical performance and quality of life between frail and robust Brazilian community-dwelling elderly women. Geriatr Gerontol Int, 16(7), 829-835.
2. Albala, C., Lera, L., Sanchez, H., Angel, B., Marquez, C., Arroyo, P., & Fuentes, P. (2017). Frequency of frailty and its association with cognitive status and survival in older Chileans. Clin Interv Aging, 12, 995-1001.
3. Lenardt MH1, Cechinel C1, Binotto MA1, Kolb Carneiro NH1, Lourenço TM1. (2016). Physical frailty and fitness of older driver. Colomb Med (Cali), 30;48(2):41-46.
4. Makizako, H., Shimada, H., Hotta, R., Doi, T., Tsutsumimoto, K., Nakakubo, S., &Makino, K. (2018). Associations of Near-Miss Traffic Incidents with Attention and Executive Function among Older Japanese Drivers. Gerontology, 64(5), 495-502.
5. Yuki, A., Otsuka, R., Tange, C., Nishita, Y., Tomida, M., Ando, F., & Shimokata, H. (2016). Epidemiology of frailty in elderly Japanese. The Journal of Physical Fitness and Sports Medicine, 5(4), 301-307.
6. Satake, S., Senda, K., Hong, Y. J., Miura, H., Endo, H., Sakurai, T., Toba, K. (2016). Validity of the Kihon Checklist for assessing frailty status. Geriatr Gerontol Int, 16(6), 709-715.
7. Fried LP1, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA. (2001). Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci, 56(3), M146-56.

2. 執筆論文　Publication of thesis　※記載した論文を添付してください。Attach all of the papers lis

論文名 1 Title					
掲載誌名 Published journal					
	年　月	巻（号）	頁 ～ 頁	言語 Language	
第1著者名 First author		第2著者名 Second author		第3著者名 Third author	
その他著者名 Other authors					
論文名 2 Title					
掲載誌名 Published journal					
	年　月	巻（号）	頁 ～ 頁	言語 Language	
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論文名 3 Title					
掲載誌名 Published journal					
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その他著者名 Other authors					
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掲載誌名 Published journal					
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掲載誌名 Published journal					
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その他著者名 Other authors					

3. 学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載

※Describe your presentation as the principal presenter in major academic meetings including general meetings or

学会名 Conference	第73回日本体力医学会大会				
演 題 Topic	複合運動プログラムが地域在住高齢者の認知機能に与える影響：認知機能水準別の検討				
開催日 date	2018 年 9 月 9 日	開催地 venue	日本・福井県		
形式 method	☐ 口頭発表 Oral ☑ ポスター発表 Poste	言語 Language	☑ 日本語 ☐ 英語 ☐ 中国語		
共同演者名 Co-presenter	藤井啓介，井上大樹，薛載勲，藤井悠也，城寶佳也，大藏倫博				
学会名 Conference					
演 題 Topic					
開催日 date	年 月 日	開催地 venue			
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Poste	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語		
共同演者名 Co-presenter					
学会名 Conference					
演 題 Topic					
開催日 date	年 月 日	開催地 venue			
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Poste	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語		
共同演者名 Co-presenter					
学会名 Conference					
演 題 Topic					
開催日 date	年 月 日	開催地 venue			
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Poste	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語		
共同演者名 Co-presenter					

4. 受賞（研究業績 Award (Research achievement)

名 称 Award name				
	国名 Country		受賞年 Year of	年 月
名 称 Award name				
	国名 Country		受賞年 Year of	年 月

5．本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your researcl

受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported	年　月 ～　年　月
受給額 Amount received	円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported	年　月 ～　年　月
受給額 Amount received	円

6．他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
奨学金名称 Scholarship	
受給期間 Supported	年　月 ～　年　月
受給額 Amount received	円

7．研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。 Attach a copy of the article described below

報道発表 Press release	□ 有　□ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞　・雑誌　・Web site　・記者発表　・その他（　　）		
発表タイトル Released title			

8．本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有　□ 無	出願国 Application	
出願内容（概要） Application contents			

9．その他 Others

指導責任者（署名） 大藏 倫博

日中笹川医学奨学金制度（学位取得コース）中間評価書
課程博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　研究者番号：G4002　作成日：2019 年 3 月 15 日

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研究先（指導教官）	筑波大学　体育系（　大藏 倫博 准教授）				
研究テーマ	地域在住高齢者におけるフレイルと交通事故発生率およびリスクとの関連				
専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	90 点　優	取得単位数 4 単位／4 単位
学生本人が行った研究の概要	身体的、精神・心理的・社会的虚弱な状態をフレイルと呼び、近年、フレイルを有する高齢者に対する健康支援の重要性が叫ばれている。当学生が博士論文テーマとしたのはフレイルを有する高齢者の交通事故リスクが高いとの仮説の下、フレイルと交通事故リスクとの関連性を多角的に検証するものである。本年度は本テーマに関する幅広い文献研究及び、それに基づき研究計画を策定した。	
総合評価	【良かった点】 ・研究テーマが明確になったため、副指導教員 2 名を決めることができた。 ・研究報告会 I を実施し、研究の進捗を発表した。 ・研究に対して、大変真面目に、かつ真摯に取り組んでいる。	
	【改善すべき点】 特に無し	
	【今後の展望】 綿密な研究計画に基づき、2019 年度は博士論文作成に必要なデータ収集をおこない、原著論文を作成する。	
学位取得見込	これまで順調に進捗しており、学位取得の可能性は極めて高いと言える。	

評価者（指導教員名）　大 藏 倫 博　印

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

Supported by 日本財団 THE NIPPON FOUNDATION

第40期　　研究者番号：G4003　　作成日：2019年3月8日

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研究先（指導教官）	東京大学大学院　医学系研究科　呼吸器外科　（　中島　淳　教授　）				
研究テーマ	肺がんに対する免疫療法の研究 Immunotherapy of Lung Cancer				
専攻種別	論文博士	□	課程博士	☑	

1．研究概要（1）

1）目的（Goal）

Cancer neoantigens represent epitopes derived from tumor-specific somatic mutations that are presented on MHCs and have emerged as promising targets for personalized cancer immunotherapy[1]. Their selective expression on tumors and lack of expression in normal somatic tissues minimize immune tolerance as well as the risk of autoimmunity. To develop effective immunotherapy strategies targeting neoantigens, we investigated the neoantigen landscape in murine lung cancer model.

2）戦略（Approach）

Whole exome sequencing and RNA-Seq analysis were conducted on Lewis lung carcinoma cells (LLC) and normal tissues of C57BL/6 mice for the identification of nonsynonymous expressed mutations. The neoepitopes were prioritized by their expression and affinity to MHC class I molecules. Naive mice were vaccinated twice with dendritic cells pulsed with neoepitopes and immune response was measured to evaluate neoepitope immunogenicity after 2 weeks. The selected high immunogenic neoepitopes were utilized for further assessment of antitumor effect.

3）材料と方法（Materials and methods）

Animal and cell lines

Six-week-old female C57BL/6 mice were purchased from Japan LC (Shizuoka, Japan). All mice were kept in a specific pathogen-free environment. The LLC cells were obtained from the ATCC. LLC cells (1*10^6) were inoculated s.c. into the flanks of C57BL/6 mice. Tumor diameter was measured twice weekly and used to calculate tumor volume(mm3) (length*width*height*π/6)

Neoantigen vaccines

We designed the neoantigen vaccines by selecting the predicted neoantigens from the exome and RNA sequencing data obtained from the LLC established tumors. Neoepitopes were prioritized from nonsynonymous coding missense mutants, where the mutant allele expression was >1 FPKM. MHC class I binding analysis was performed using NetMHCpan v2.8.

Immunogenicity

Dendritic cells were cultured and maturated from C57BL/6 mice bone marrow 2 weeks beforehand. Neoepitopes were pulsed to mature dendritic cells for 2 hours before injection with a concentration of 1ug/ml. Naive mice were vaccinated twice on 2-week intervals, and immune responses were measured 2 weeks after second vaccination with intracellular staining and ELISA .

1. 研究概要（2）

3）材料と方法（Materials and methods）

LLC tumor challenge experiments
Dendritic cells were prepared as referred before. 2 weeks later, we implanted mice with the LLC cell line subcutaneously (100,0000 cells in the flank), and measured tumor size twice a week by caliper. Mice were euthanized when tumor size reached 2000 mm3.

4）実験結果（Results）

We identified 2536 Missense mutations in LLC-1 totally. Of these, 856 were expressed (FPKM≧1). MHC class I binding affinity of these mutated peptide is estimated by netMHCpan 2.8. First, 60 mutated peptides with netMHC score (IC50) <200 nM were selected as high affinity peptides. In addition, 68 mutated peptides with IC50>200 nM and the ratio of wild to corresponding mutated type peptide in netMHC score>10 were included as moderate affinity peptides.
C57BL/6 mice were immunized with dendritic cells pulsed with these mutated peptides. Antigenicity of peptides were evaluated by IFN-γ production of splenocytes ex vivo and after culture with corresponding peptides in vitro. So far 60 high affinity neoepitopes and 68 moderate affinity neoepitopes were analyzed. Totally, 22 mutated peptides induced neoepitope-specific response. However, only 7 neoepitopes exhibited weak antitumor effect without statistical significance.

5）考察（Discussion）

IFN-γ CD8+ T cells induced by neoepitope peptides could be detected in splenocytes, which are inconsistent with the fact that the epitopes were selected in silico for high MHC I binding affinity. Positive antitumor response treated with single neoepitope peptide vaccine were very weak[2].
Our study suggests that all mutated peptides are not equally immunogenic. Selection or prioritization of neoantigen that can induce anti-tumor response is critical for the development of neoantigen-targeting immunotherapy[3].
In our following experiment, multiple neoepitopes in combination with checkpoint inhibitors will be performed, since high likelihood of tumor escapes and exhausted T cells exist[4, 5].

6）参考文献（References）

[1]Sahin U, Tureci O. Personalized vaccines for cancer immunotherapy. Science 2018;359:1355-60.
[2]Duperret EK, Perales-Puchalt A, Stoltz R, Hiranjith GH, Mandloi N, Barlow J et al. A Synthetic DNA, Multi-Neoantigen Vaccine Drives Predominately MHC Class I CD8(+) T-cell Responses, Impacting Tumor Challenge. Cancer Immunol Res 2019;7:174-82.
[3]Srivastava PK. Neoepitopes of Cancers: Looking Back, Looking Ahead. Cancer Immunol Res 2015;3:969-77.
[4]Brennick CA, George MM, Corwin WL, Srivastava PK, Ebrahimi-Nik H. Neoepitopes as cancer immunotherapy targets: key challenges and opportunities. Immunotherapy-Uk 2017;9:361-71.
[5]Hu Z, Ott PA, Wu CJ. Towards personalized, tumour-specific, therapeutic vaccines for cancer. Nat Rev Immunol 2018;18:168-82.

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title	Low truncal muscle area on chest computed tomography: a poor prognostic factor for the cure of early-stage non-small-cell lung cancer					
掲載誌名 Published journal	European Journal of Cardio-Thoracic Surgery					
	2019 年 3 月	55 巻（号）	414 頁 ～ 420 頁	言語 Language	English	
第1著者名 First author	Changbo Sun	第2著者名 Second author	Masaki Anraku	第3著者名 Third author	Takahiro Karasaki	
その他著者名 Other authors	Hideki Kuwano Kazuhiro Nagayama Jun-Ichi Nitadori Masaaki Sato Jun Nakajima					
論文名 2 Title						
掲載誌名 Published journal						
	年 月	巻（号）	頁 ～ 頁	言語 Language		
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 3 Title						
掲載誌名 Published journal						
	年 月	巻（号）	頁 ～ 頁	言語 Language		
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 4 Title						
掲載誌名 Published journal						
	年 月	巻（号）	頁 ～ 頁	言語 Language		
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 5 Title						
掲載誌名 Published journal						
	年 月	巻（号）	頁 ～ 頁	言語 Language		
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						

3. 学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載し

※Describe your presentation as the principal presenter in major academic meetings including general meetings c

学会名 Conference	第112回臨床呼吸生理研究会学術集会			
演 題 Topic	Impact of preoperative pectoralis muscle quantity and density on outcome after complete resectionof non-small cell lung cancer			
開催日 date	2018 年 6 月 30 日	開催地 venue	Tokyo	
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Post	言語 Language	☐ 日本語 ☑ 英語 ☐ 中国語	
共同演者名 Co-presenter				
学会名 Conference	The 16th Annual Meeting of Japan Research Association for Immunotherapeutics			
演 題 Topic	The neoantigen landscape of murine lung cancer LLC-1 model			
開催日 date	2019 年 2 月 23 日	開催地 venue	Tokyo	
形式 method	☐ 口頭発表 Oral ☑ ポスター発表 Post	言語 Language	☐ 日本語 ☑ 英語 ☐ 中国語	
共同演者名 Co-presenter				
学会名 Conference				
演 題 Topic				
開催日 date	年 月 日	開催地 venue		
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Post	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語	
共同演者名 Co-presenter				
学会名 Conference				
演 題 Topic				
開催日 date	年 月 日	開催地 venue		
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Post	言語 Language	☐ 日本語 ☐ 英語 ☐ 中国語	
共同演者名 Co-presenter				

4. 受賞（研究業績）Award (Research achievement)

名 称 Award name	第112回臨床呼吸生理研究会奨励賞			
	国名 Country	日本	受賞年 Year of	2018 年 6 月
名 称 Award name				
	国名 Country		受賞年 Year of	年 月

5．本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch

受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported	年　月 ～　年　月
受給額 Amount received	円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported	年　月 ～　年　月
受給額 Amount received	円

6．他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
奨学金名称 Scholarship	
受給期間 Supported	年　月 ～　年　月
受給額 Amount received	円

7．研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。 Attach a copy of the article described below

報道発表 Press release	□ 有　□ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞　・雑誌　・Web site　・記者発表　・その他（　　）		
発表タイトル Released title			

8．本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有　□ 無	出願国 Application	
出願内容（概要） Application contents			

9．その他 Others

指導責任者（署名）　中島 淳

日中笹川医学奨学金制度(学位取得コース)中間評価書
課程博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　研究者番号：G4003　作成日 ：2019 年 3 月13日

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研究テーマ	肺がんに対する免疫療法の研究 Immunotherapy of Lung Cancer				
専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	優 学業成績係数=	取得単位数 取得単位数＝８／取得すべき単位総数＝３２
学生本人が行った研究の概要	肺癌におけるがん免疫の機序に関する基礎的研究ならびに新たながん免疫治療の方法に関する研究を行った。Lewis lung carcinoma cell line (LLC)をマウスに移植して肺癌モデルを作成した。LLCの全エクソーム解析、RNA-Seq 解析を行い MHC 親和性のある癌特異的な neopeptide ライブラリを作成した。この neopeptide によって感作された樹状細胞をマウスに接種し、移植されたＬＬＣ細胞の発育阻害を検討した。	
総合評価	【良かった点】 LLC 細胞株の全エクソーム解析および RNA-Seq 解析を比較的短時間で、初年度で冠水することができたこと、またＬＬＣのマウスへの接種や、樹状細胞の neopeptide による刺激などの基本的な実験手技を十分に取得されたこと	
	【改善すべき点】 研究の進捗状況については特に問題なし。結果に関しては、現時点では、樹状細胞ワクチンだけでは十分な抗癌効果が得られておらず、さらに来年度にも検討が必要である。	
	【今後の展望】 樹状細胞ワクチンに加え、抗癌作用を強化するため免疫チェックポイント阻害剤を併用した実験系の開発を行う。	
学位取得見込	大学院生期間内（4 年間もしくはそれ以内)に取得可能であると判断する。	
評価者（指導教官名）	中島 淳　印	

European Journal of Cardio-Thoracic Surgery 55 (2019) 414–420
doi:10.1093/ejcts/ezy324 Advance Access publication 4 October 2018

ORIGINAL ARTICLE

Cite this article as: Sun C, Anraku M, Karasaki T, Kuwano H, Nagayama K, Nitadori J-I *et al.* Low truncal muscle area on chest computed tomography: a poor prognostic factor for the cure of early-stage non-small-cell lung cancer. Eur J Cardiothorac Surg 2019;55:414–20.

Low truncal muscle area on chest computed tomography: a poor prognostic factor for the cure of early-stage non-small-cell lung cancer†

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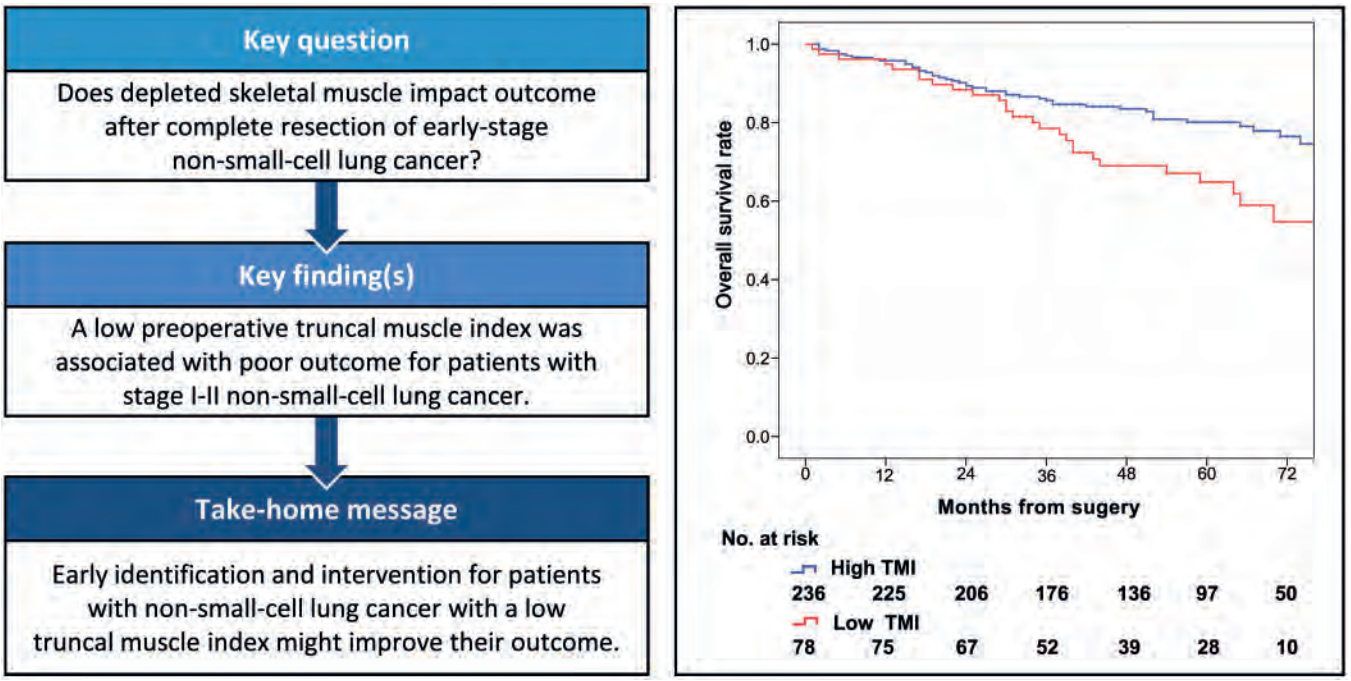
Received 8 June 2018; received in revised form 12 August 2018; accepted 27 August 2018



Abstract

OBJECTIVES: Depletion in skeletal muscle is closely associated with limited physical ability and high mortality. In this study, we evaluated the prognostic significance of skeletal muscle depletion in patients with early-stage non-small-cell lung cancer.

METHODS: A retrospective analysis of patients with pathological stages I–II lung cancer, who underwent curative resection between 2009 and 2013, was conducted. The truncal muscle index (TMI) (area/height^2) at the first lumbar vertebral level was measured by preoperative axial computed tomography. Overall survival and recurrence-free survival were compared between the lowest gender-specific quartile of the TMI and the other quartiles.

†Presented at the 31st Annual Meeting of the European Association for Cardio-Thoracic Surgery, Vienna, Austria, 7–11 October 2017.

RESULTS: A total of 314 subjects were included in the study. The cumulative 5-year recurrence-free and overall survival rates were significantly shorter in patients with lower TMIs (69% vs 83.5%, $P = 0.028$; 64.8% vs 80.1%, $P = 0.003$, respectively). In multivariable models, the TMI was identified as an independent prognostic factor for overall survival ($P = 0.017$, hazard ratio 1.84, 95% confidence interval 1.12–3.05), after adjusting for age, gender, preoperative serum albumin, carcinoembryonic antigen, neutrophil to lymphocyte ratio and pathological stage.

CONCLUSIONS: A low preoperative TMI was associated with a poor postoperative outcome in patients with early-stage non-small-cell lung cancer. This factor may be included in the preoperative assessment of patients, for whom surgical intervention is considered.

Keywords: Skeletal muscle depletion • Truncal muscle index • Early-stage non-small-cell lung cancer • Prognostic factor

INTRODUCTION

Lung cancer is one of the most frequently diagnosed cancers and one of the leading causes of cancer mortality in the world [1]. The overall survival (OS) of patients with early-stage non-small-cell lung cancer (NSCLC; stages I–II) is distinctly better than that of patients with advanced lung cancer. However, the postoperative prognosis is poor for some patients with early-stage NSCLC (stages I–II). It refers to both cancer-specific factors and individual patient characteristics. Poor survival rates due to pathological subtypes or systemic inflammation were reported in patients with early-stage NSCLC (stages I–II) undergoing curative surgery [2–4].

Sarcopenia—the loss of muscle mass and function—has been clinically identified as a poor predictor [5, 6]. Sarcopenia contributes to functional decline, disability, injury and mortality. The link between sarcopenia and poor prognosis was first reported in patients with non-malignant diseases and in geriatric populations. Recently, the clinical importance of sarcopenia has also been increasingly recognized in oncological patients [7, 8]. Low skeletal muscle—a key and objective component of sarcopenia—was investigated, and the results indicated a close association with limited physical ability and high mortality in advanced cancers [9]. However, the correlation between low skeletal muscle and the prognosis of early-stage NSCLC is not well understood.

The present study investigated the truncal muscle area on chest computed tomography (CT) to determine the impact of skeletal muscle mass depletion on the prognosis of patients with stages I–II NSCLC undergoing curative surgery.

MATERIALS AND METHODS

Patients

A retrospective analysis of patients with stages I–II NSCLC who underwent lobectomy and mediastinal lymph node dissection at the University of Tokyo Hospital (Tokyo, Japan) from January 2009 to December 2013 was conducted. Eligible patients had pathological stages I–II NSCLC following surgery. Preoperative (i.e. within 90 days prior to surgery) chest CT images of the study population were available for review (Fig. 1).

Data collection

Data collected from inpatient and outpatient records included demographics [age, gender, body mass index (BMI)], blood count and serum biochemical data for a week prior to the operation [leukocytes, neutrophils, lymphocytes, serum albumin (Alb) and C-reactive protein], tumour-specific data [carcinoembryonic antigen (CEA)], postoperative complications based on the extended Clavien–Dindo classification (see Supplementary Material, Table S1) [10], pathological data [histology and tumour, node and metastasis (TNM) staging according to the 7th UICC-TNM classification] and survival data including recurrence-free survival (RFS) and OS. RFS was defined as the period from the date of surgery to that of first recurrence or death. OS was defined as the period from the date of surgery to that of death (by any cause) or lost follow-up. All patients provided written informed consent prior to the analyses.

Chest CT examinations were performed using a 64-detector CT (Aquilion ONE Vision Edition Aquilion PRIME, Toshiba, Japan or Discovery CT750 HD, General Electric, USA) with a 5-mm slice thickness. The patients were requested to maintain a supine position with raised arms and were asked to hold their breath at deep inspiration during the chest CT examination. The truncal muscle area at the first lumbar vertebral level (L1) was identified on a chest CT scan taken prior to surgery (Fig. 2A). The truncal muscle area, comprising the paraspinal muscles and chest–abdominal wall muscles, was plotted at the transverse process level of L1 [8] (Fig. 2B). The SYNAPSE VINCENT (Fujifilm Medical, Tokyo, Japan) image analysis software was used to define the skeletal muscle area semiautomatically. The skeletal muscle area was identified and quantified based on Hounsfield unit thresholds (−29 to +150) in square millimetres (mm^2) (Fig. 2C).

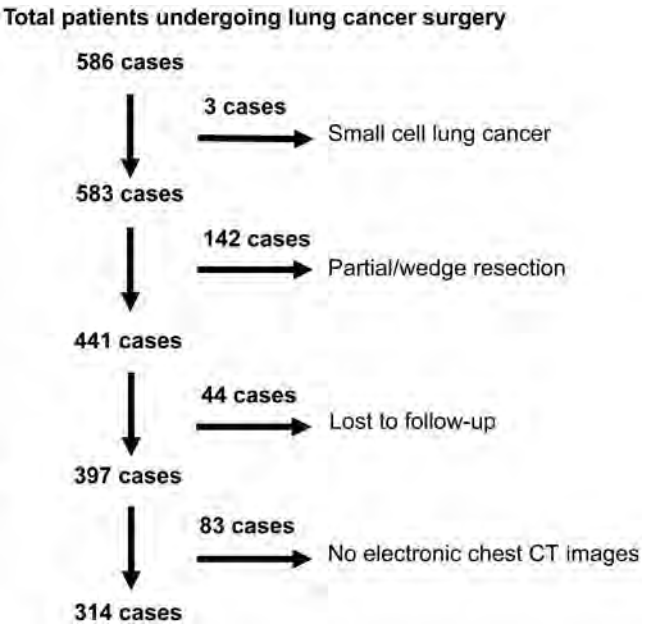


Figure 1: The study cohort. CT: computed tomography.

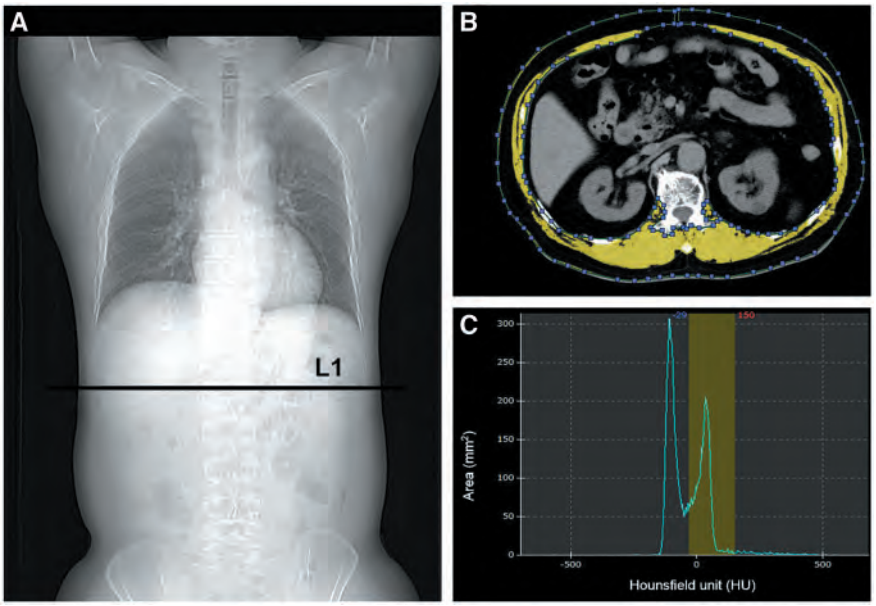


Figure 2: (**A**) Muscle area calculations at the process of the first lumbar vertebra (L1). (**B**) Truncal muscles consist of paraspinal muscles and chest–abdominal wall muscles at L1 (yellow area). (**C**) Truncal muscle area was identified and quantified based on Hounsfield unit thresholds (−29 to +150) in square millimetres (mm^2), with the exception of right wave indicating fat tissue area.

For the calculation of the truncal muscle index (TMI), the muscle area was divided by the square of height (m^2). The lowest quartile cut-off values of the TMI were used to divide patients into the low-TMI group and the high-TMI group in the study. Image analysis was performed without access to information on surgical outcomes to ensure unbiased measurements and calculations.

Statistical analysis

All statistical analyses were performed using the SPSS, version 22.0, software (IBM Inc., Armonk, NY, USA). All data are expressed as the median (interquartile range), with exception of age, which are presented as the mean [±standard deviation (SD)]. Gender and smoking status are showed as categorical data. Differences between groups were analysed using the Mann–Whitney *U*-test for continuous variables and Pearson's χ^2 test for categorical data. RFS and OS curves were plotted using the Kaplan–Meier method, and differences were compared using the log-rank test. Cox regression survival analysis was performed for the following factors: age, smoking history, BMI, Alb, neutrophil to lymphocyte ratio (NLR), CEA, pathological stage (p-TNM) and TMI. Variables with *P*-value <0.05 in the univariable analysis were also used for the multivariable analysis. Differences with *P*-value <0.05 were considered statistically significant.

RESULTS

Patient characteristics

The clinical and pathological characteristics of 314 patients with p-stages I–II NSCLC with a mean age of 68.1 ± 10.6 years included in this study are listed in Table 1. Of them, the majority had adenocarcinoma (227 patients, 72.3%), followed by squamous carcinoma (63 patients, 20.1%), large cell carcinoma (4 patients, 1.2%) and other NSCLCs (20 patients, 6.4%), as confirmed by histology. A total of 59 recurrences and 77 deaths were reported in the follow-up period.

The median TMI for men was 41.2 cm^2/m^2, whereas for women, the value was 33.4 cm^2/m^2. The indices were significantly higher in men than women ($P < 0.001$). The correlation between BMI and TMI was significant (Pearson's $r = 0.574$, $P < 0.001$). The median serum Alb, NLR and CEA were 4 g/dl, 2.2 and 4.3 μg/l, with Alb, NLR, CEA data of 1 patient, 35 patients and 6 patients missing, respectively. Accordingly, 274 patients were analysed in the multivariable analyses.

Clinicopathological factors and survival analysis

The TMI was significantly higher in men than in women ($P < 0.001$). Thus, patients were divided into the low-TMI and high-TMI groups based on the gender-specific lowest quartile cut-off values of the TMI (38 cm^2/m^2 for men and 29.6 cm^2/m^2 for women). As a result, 236 and 78 cases are included in the low-TMI and high-TMI groups, respectively. The comparison of clinicopathological factors between the low-TMI and high-TMI groups is shown in Table 1. Patients with the low TMI had a significantly lower BMI (median 20.1 vs 23 kg/m^2, respectively, $P < 0.001$) and Alb (<4 vs ≥4 g/dl, respectively, $P = 0.046$) than those with the high TMI. Additional factors such as age, smoking history, CEA, NLR, pathology distribution and p-TNM were not significantly different between the low-TMI and high-TMI groups. To identify any factors associated with low TMI, we performed the univariable analyses with age, smoking status, BMI, Alb, CEA, NLR and p-TNM. BMI, NLR and Alb were significant risk factors ($P < 0.001$, $P = 0.007$ and $P = 0.024$, respectively). The factors including BMI, Alb, NLR, age and p-TNM with *P*-value <0.25 in the univariable analyses were evaluated in a multivariable logistic analysis. Only BMI was a significant risk factor for TMI [$P < 0.001$, hazard ratio 1.49, 95% confidence interval (CI) 1.30–1.71].

Table 1: Clinical characteristics of the low TMI and high TMI groups

Category	Low TMI group ($n = 78$)	High TMI group ($n = 236$) (cm^2/m^2)	*P*-value
Gender, *n* (%)			0.89
Male	46 (59.0)	137 (58.1)	
Female	32 (41.0)	99 (41.9)	
Age (years), mean ± SD	72 ± 8.9	67 ± 11	0.073
<65	21 (26.9)	78 (33.1)	
≥65	57 (73.1)	158 (66.9)	
Smoking history, *n* (%)			0.20
Non-smoker	25 (32.1)	95 (40.3)	
Smoker	53 (67.9)	141 (59.7)	
BMI (kg/m^2), median (IQR)	20.1 (18.8–21.6)	23 (21–24.9)	<0.001
<18.5[a]	15 (19.2)	7 (3.0)	
18.5–25[b]	60 (76.9)	173 (73.3)	
≥25.0[c]	3 (3.8)	56 (23.7)	
NLR, median (IQR)	2.4 (1.7–3.6)	2.2 (1.7–3.2)	0.20
≤3	47 (70.1)	153 (72.2)	
>3	20 (29.9)	59 (27.8)	
Alb (g/dl), median (IQR)	4 (3.7–4.2)	4.1 (3.8–4.3)	0.046
<4	37 (48.1)	89 (37.7)	
≥4	40 (51.9)	147 (62.3)	
CEA (μg/l), *n* (%)			0.50
≤5	46 (59.7)	148 (64.1)	
<5	31 (40.3)	83 (35.9)	
Postoperative complication, *n* (%)			0.82
Present	13 (16.7)	42 (17.8)	
Absent	65 (83.3)	194 (82.2)	
Pathology (NSCLC), *n* (%)			0.81
Adenocarcinoma	59 (75.6)	168 (71.2)	
Squamous carcinoma	15 (19.2)	48 (20.3)	
Large cell carcinoma	1 (1.3)	4 (1.7)	
Others	3 (3.8)	16 (6.8)	
p-TNM (7th edition), *n* (%)			0.78
Stage 1	59 (75.6)	195 (82.6)	
Stage 2	19 (24.4)	41 (17.4)	

P is shown as $P < 0.001$ if the actual *P*-value was <0.001.

[a]Underweight.

[b]Normal weight.

[c]Overweight and obesity.

Alb: albumin; BMI: body mass index; CEA: carcinoembryonic antigen; IQR: interquartile range; NLR: neutrophil to lymphocyte ratio; NSCLC: non-small-cell lung cancer; SD: standard deviation; TMI: truncal muscle index (cm^2/m^2); TNM: tumour, node and metastasis.

The RFS and OS Kaplan–Meier curves for patients with the low TMI and high TMI are shown in Fig. 3. RFS was significantly lower in patients with the low TMI compared with that of patients with high TMI (5-year RFS 69% vs 83.5%, respectively, $P = 0.028$, Fig. 3A). Similarly, OS was significantly different between the low-TMI group and high-TMI group (5-year OS 64.8% vs 80.1%, respectively, $P = 0.003$, Fig. 3B). The results of the Cox regression survival analysis of RFS and OS are shown in Tables 2 and 3. The univariable analysis of RFS identified the following significant prognostic factors in patients: smoking history, NLR, Alb, CEA, p-TNM and TMI. However, in the multivariable analysis, only NLR and p-TNM were shown to be the independent prognostic factors for RFS. The univariable analysis of OS indicated that smoking history, NLR, albumin, CEA, p-TNM and TMI were associated with postoperative prognosis. The multivariable analysis demonstrated that the TMI was an independent prognostic factor (hazard ratio 1.84, 95% CI 1.12–3.05; $P = 0.017$), in addition to p-TNM, NLR and Alb.

DISCUSSION

To our knowledge, this is the first study that investigated the impact of skeletal muscle volume represented by truncal muscle cut surface on the outcome of patients with early-stage NSCLC (stages I–II) who underwent curative surgery. The impact of depletion of the cross-sectional truncal muscle area at L1 (TMI) on outcomes was assessed using chest CT. The decrease in the TMI is an independent prognostic factor with an 1.8-fold increased risk of death in patients.

CT, which is routinely performed as a pretreatment staging assessment of patients with cancer, is widely used to evaluate skeletal muscle [11]. The identification and quantification of the skeletal muscle area on CT is recommended due to its precise differentiation between muscle, fat and other tissues. The single cross-sectional area of muscle at the third lumbar vertebra (L3) is referred to as a good modality, as it linearly relates to total body skeletal muscle mass on abdominal CT [12]. However, chest CT rarely extends to the L3 level. The implementation of muscle measurement and further progress in the field of surgical lung cancer care are severely hampered due to the lack of a standardized and efficient approach [13, 14]. In healthy subjects, examination of the muscle area at L1 via chest CT showed high correlation with the total body skeletal muscle mass [15]. As a result, we investigated truncal muscle area on the L1 of chest CT to evaluate the clinical impact on outcome of stages I–II NSCLC patients.

Low truncal muscle is a poor independent prognostic factor after complete resection in patients with early stage NSCLC. Low skeletal muscle, associated with a risk of adverse outcomes such as physical disability, poor quality of life and death, plays an important role in predicting chemotherapeutic toxicity and treatment outcomes in certain advanced cancers [7, 8]. Recently, clinical studies demonstrated that low skeletal muscle prior to surgery negatively impacts survival of patients with resectable gastrointestinal, hepatopancreatobiliary, colorectal and endometrial malignancies [16–19]. However, few studies have focused on the impact of skeletal muscle mass on prognosis in operable NSCLC due to the lack of an appropriate method for the measurement of skeletal muscle using chest CT. Suzuki *et al.* [20] reported that sarcopenia at the L3 level on abdominal CT was associated with poor outcome in a small sample of patients with completely resected early-stage NSCLC. The analysis of the present study indicated that the TMI on chest CT may be a practical and valuable method for the preoperative assessment of skeletal muscle mass in early-stage NSCLC (stages I–II) patients undergoing curative surgery.

It appeared to be multifactorial for the low TMI in early-stage NSCLC patients. It is well acknowledged that the recurrence of NSCLC after complete resection was relatively higher in the first 2 years [2]. The RFS and OS curves of the high- and low-TMI groups in the present study showed no distinct difference in the first 2 years. However, divergences were almost synchronously observed from the third year in both survival curves, although the significance of TMI was not independent in the multivariable

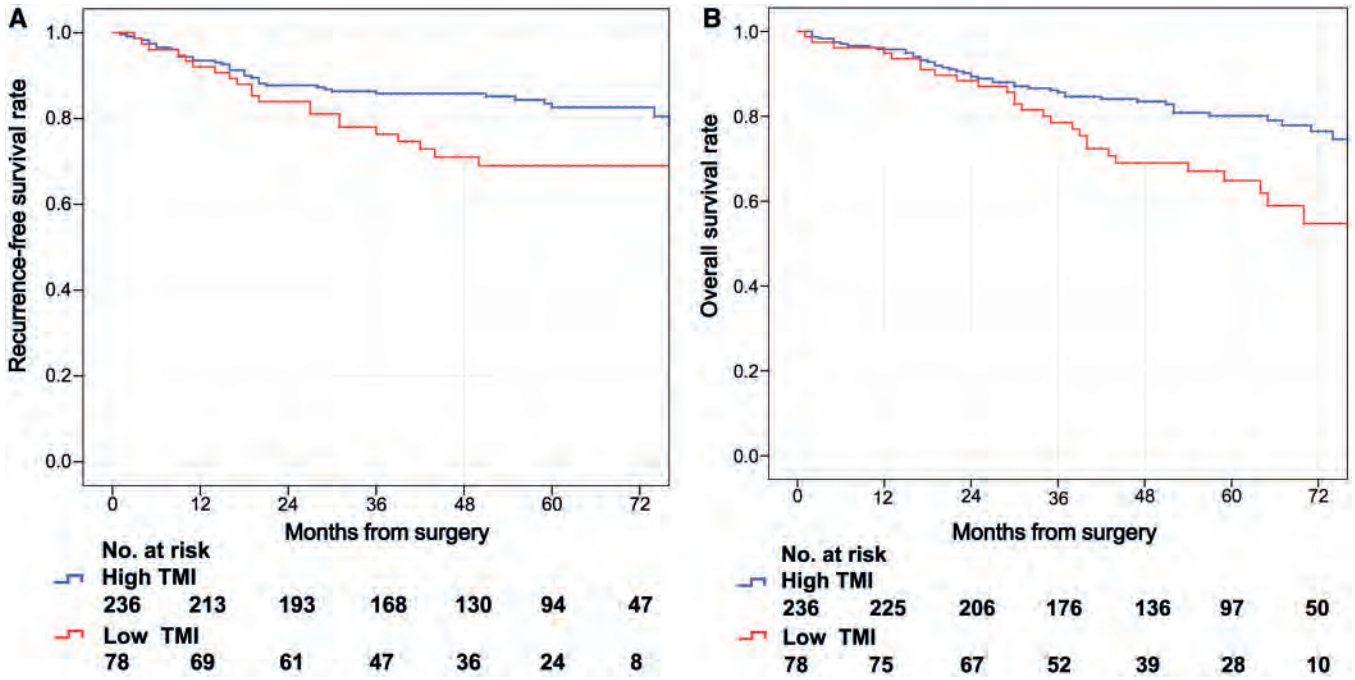


Figure 3: Survival curves of subgroups divided by the lowest quartile and the rest of the TMI. TMI is significantly prognostic for (**A**) recurrence-free survival and (**B**) overall survival. TMI: truncal muscle index.

Table 2: Results of univariable and multivariable analyses of recurrence-free survival ($n = 274$)

Variables	Univariable			Multivariable		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Male gender	1.45	0.85–2.47	0.17			
Age ≥65 years	1.7	0.93–3.10	0.084			
Current/ex-smoker	1.79	1.02–3.16	0.043	1.8	0.97–3.34	0.064
BMI <18.5 kg/m^2	1.51	0.65–3.52	0.34			
NLR >3	2.59	1.51–4.42	0.001	2.08	1.17–3.70	0.013
Alb ≤4	2.11	1.26–3.52	0.004	1.57	0.88–2.81	0.13
CEA >5	1.74	1.04–2.92	0.036	1.09	0.63–1.91	0.76
p-stage II (7th edition)	4.87	2.90–8.17	<0.001	4.09	2.35–7.12	<0.001
Low truncal muscle index	1.81	1.06–3.09	0.029	1.42	0.80–2.52	0.23

Alb: albumin; BMI: body mass index; CEA: carcinoembryonic antigen; CI: confidence interval; HR: hazard ratio; NLR: neutrophil to lymphocyte ratio.

Table 3: Results of univariable and multivariable analyses of overall survival ($n = 274$)

Variables	Univariable			Multivariable		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Male gender	1.77	1.09–2.87	0.021	1.2	0.65–2.21	0.56
Age ≥65 years	1.98	1.14–3.44	0.016	1.16	0.63–2.13	0.64
Smoker	2.22	1.32–3.74	0.003	1.57	0.81–3.04	0.19
BMI <18.5 kg/m^2	1.05	0.42–2.60	0.92			
NLR >3	3.24	2.02–5.21	<0.001	2.35	1.38–4.00	0.002
Alb ≤4	3.03	1.90–4.84	<0.001	2	1.18–3.39	0.01
CEA >5	1.95	1.24–3.08	0.004	1.27	0.78–2.09	0.34
p-stage II (7th edition)	4.01	2.52–6.37	<0.001	3.44	2.08–5.68	<0.001
Low truncal muscle index	2	1.26–3.18	0.002	1.84	1.12–3.05	0.017

Alb: albumin; BMI: body mass index; CEA: carcinoembryonic antigen; CI: confidence interval; HR: hazard ratio; NLR: neutrophil to lymphocyte ratio.

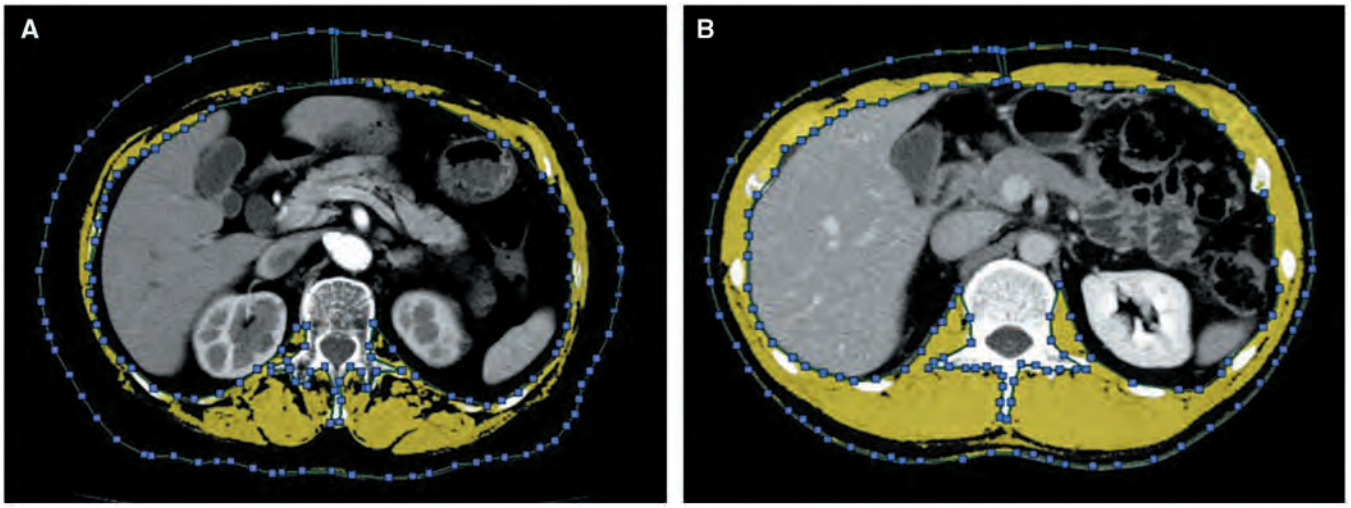


Figure 4: The body mass indices of 2 patients (**A** and **B**) were almost identical (23.2 and 23.5 kg/m^2). However, the muscle areas were different. The truncal muscle indices of these patients were 29 and 48, respectively.

analysis of RFS. Accordingly, low truncal muscle may be caused by tumour-related and non-tumour-related factors such as ageing, metabolic disorder, decreased physical activity, increased risk of cardiovascular disease and therapy-resistant metabolic diseases [9, 21, 22]. This was reflected on poor long-time outcome of patients with low truncal muscle had in early NSCLC.

Some evidence indicated that improving physical activity and nutritional intervention are a promising cancer therapy [23]. However, improving the skeletal muscle prior to surgery was impractical given the urgent need of resection for early NSCLC. The present study showed that low skeletal muscle was a risk factor for prognosis and provided an objective method to evaluate this risk factor at the time of treatment. Comprehensive information with well-planned care could be provided to the patients with poor skeletal muscle prior to surgery. More positive supports such as physical exercise and nutritional intervention for low skeletal muscle patients may be needed even after the treatment, because the survival curves of the low-TMI and high-TMI groups diverged from the third year after the surgery (Fig. 3B). Future prospective studies are needed to clarify whether interventions for increasing skeletal muscle can improve postoperative outcomes in patients with NSCLC.

The relationship between BMI and postoperative outcomes in cancer patients has been a subject of controversy. A previous study reported that increased body weight was associated with increased death rates in all cancers combined [24]. In contrast, obesity in patients with lung cancer has been linked to improved postoperative outcomes in another study [25]. BMI did not show a significant correlation with prognosis, although it was an independent risk factor for TMI in the present study. Instead, body composition (i.e. TMI) was suggested to be a prognostic factor. The difference in the prognostic value of TMI versus BMI may be partly explained by the fact that individuals with similar BMIs may have different body compositions (e.g. more fat or muscle in a patient than in another; Fig. 4) (see Supplementary Material, Fig. S1).

In our study, sex-specific quartile values rather than specific cut-off values were used to define the levels of skeletal muscle depletion. The definition of sarcopenia is an appendicular skeletal muscle index of more than 2 SD below that of healthy adults [5]. However, the actual prevalence of sarcopenia in Japanese patients is still unclear, and the skeletal muscle indices are vary with ethnicity. Therefore, the method of sex-specific quartiles was commonly applied to evaluate the skeletal muscle depletion.

Limitations

The present study is characterized by 2 main limitations. Firstly, this was a retrospective study with a limited patient sample size in a single institution. It is essential that these data are confirmed by large-scale population-based prospective studies. Secondly, this study was based on a single time point of chest CT prior to surgery. The changes in muscle mass postoperatively ought to be evaluated further in future studies. In addition, not only morphological muscle assessment but also sarcopenia-related function evaluation is of interest.

CONCLUSION

In conclusion, the findings of this study demonstrated that truncal skeletal muscle is an independent prognostic factor in patients with stages I–II NSCLC following curative surgery. This factor may be included in the preoperative assessment in patients with early-stage NSCLC, for whom surgical intervention is considered.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EJCTS* online.

ACKNOWLEDGEMENTS

The authors thank Yukari Uemura for the statistical review of the manuscript.

Funding

C.S. received Japan-China Sasakawa Medical Fellowship from the Sasakawa Memorial Health Foundation.

Conflict of interest: none declared.

REFERENCES

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.

[2] Goldstraw P, Chansky K, Crowley J, Rami-Porta R, Asamura H, Eberhardt WE *et al*. The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. J Thorac Oncol 2016;11:39–51.

[3] Maeda R, Yoshida J, Ishii G, Hishida T, Nishimura M, Nagai K. Prognostic impact of histology on early-stage non-small cell lung cancer. Chest 2011;140:135–45.

[4] Alifano M, Mansuet-Lupo A, Lococo F, Roche N, Bobbio A, Canny E *et al*. Systemic inflammation, nutritional status and tumor immune microenvironment determine outcome of resected non-small cell lung cancer. PLoS One 2014;9:e106914.

[5] Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F *et al*. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. Age Ageing 2010;39:412–23.

[6] Muscaritoli M, Anker SD, Argiles J, Aversa Z, Bauer JM, Biolo G *et al*. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr 2010;29:154–9.

[7] Martin L, Birdsell L, Macdonald N, Reiman T, Clandinin MT, McCargar LJ *et al*. Cancer cachexia in the age of obesity: skeletal muscle depletion is a powerful prognostic factor, independent of body mass index. J Clin Oncol 2013;31:1539–47.

[8] Prado CM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, Martin L *et al*. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. Lancet Oncol 2008;9:629–35.

[9] Joglekar S, Nau PN, Mezhir JJ. The impact of sarcopenia on survival and complications in surgical oncology: a review of the current literature. J Surg Oncol 2015;112:503–9.

[10] Katayama H, Kurokawa Y, Nakamura K, Ito H, Kanemitsu Y, Masuda N *et al*. Extended Clavien-Dindo classification of surgical complications: Japan Clinical Oncology Group postoperative complications criteria. Surg Today 2016;46:668–85.

[11] Mourtzakis M, Prado CM, Lieffers JR, Reiman T, McCargar LJ, Baracos VE. A practical and precise approach to quantification of body composition in cancer patients using computed tomography images acquired during routine care. Appl Physiol Nutr Metab 2008;33:997–1006.

[12] Shen W, Punyanitya M, Wang Z, Gallagher D, St-Onge MP, Albu J *et al*. Total body skeletal muscle and adipose tissue volumes: estimation from a single abdominal cross-sectional image. J Appl Physiol 2004;97:2333–8.

[13] Rier HN, Jager A, Sleijfer S, Maier AB, Levin MD. The prevalence and prognostic value of low muscle mass in cancer patients: a review of the literature. Oncologist 2016;21:1396–409.

[14] Collins J, Noble S, Chester J, Coles B, Byrne A. The assessment and impact of sarcopenia in lung cancer: a systematic literature review. BMJ Open 2014;4:e003697.

[15] Kim EY, Kim YS, Park I, Ahn HK, Cho EK, Jeong YM *et al*. Evaluation of sarcopenia in small-cell lung cancer patients by routine chest CT. Support Care Cancer 2016;24:4721–6.

[16] Fujiwara N, Nakagawa H, Kudo Y, Tateishi R, Taguri M, Watadani T *et al*. Sarcopenia, intramuscular fat deposition, and visceral adiposity independently predict the outcomes of hepatocellular carcinoma. J Hepatol 2015;63:131–40.

[17] Miyamoto Y, Baba Y, Sakamoto Y, Ohuchi M, Tokunaga R, Kurashige J *et al*. Sarcopenia is a negative prognostic factor after curative resection of colorectal cancer. Ann Surg Oncol 2015;22:2663–8.

[18] Kuroki LM, Mangano M, Allsworth JE, Menias CO, Massad LS, Powell MA *et al*. Pre-operative assessment of muscle mass to predict surgical complications and prognosis in patients with endometrial cancer. Ann Surg Oncol 2015;22:972–9.

[19] Ninomiya G, Fujii T, Yamada S, Yabusaki N, Suzuki K, Iwata N *et al*. Clinical impact of sarcopenia on prognosis in pancreatic ductal adenocarcinoma: a retrospective cohort study. Int J Surg 2017;39:45–51.

[20] Suzuki Y, Okamoto T, Fujishita T, Katsura M, Akamine T, Takamori S *et al*. Clinical implications of sarcopenia in patients undergoing complete resection for early non-small cell lung cancer. Lung Cancer 2016;101:92–7.

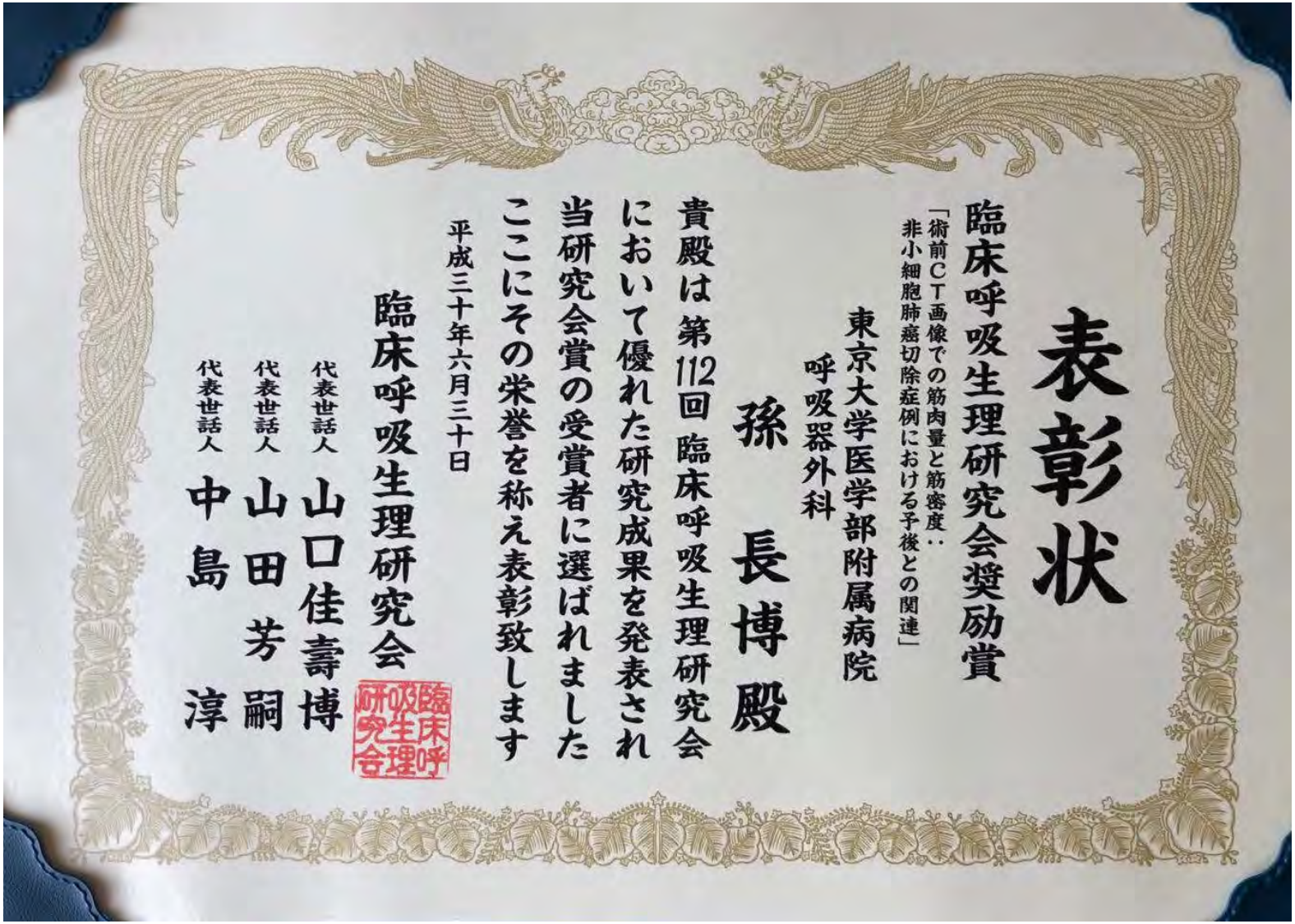
[21] Ahima RS, Lazar MA. Physiology. The health risk of obesity–better metrics imperative. Science 2013;341:856–8.

[22] Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J *et al*. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering—prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med 2008;168:1617–24.

[23] Bade BC, Thomas DD, Scott JB, Silvestri GA. Increasing physical activity and exercise in lung cancer: reviewing safety, benefits, and application. J Thorac Oncol 2015;10:861–71.

[24] Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 2003;348:1625–38.

[25] Attaran S, McShane J, Whittle I, Poullis M, Shackcloth M. A propensity-matched comparison of survival after lung resection in patients with a high versus low body mass index. Eur J Cardiothorac Surg 2012;42:653–8.



表彰状

臨床呼吸生理研究会奨励賞

「術前CT画像での筋肉量と筋密度：
非小細胞肺癌切除症例における予後との関連」

東京大学医学部附属病院
呼吸器外科

孫　長博　殿

貴殿は第112回臨床呼吸生理研究会
において優れた研究成果を発表され
当研究会賞の受賞者に選ばれました
ここにその栄誉を称え表彰致します

平成三十年六月三十日

臨床呼吸生理研究会

代表世話人　山口佳壽博
代表世話人　山田芳嗣
代表世話人　中島淳

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

Supported by 日本財団 THE NIPPON FOUNDATION

第40期　　研究者番号：G4004　　作成日：2019年3月 10 日

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所属機関(役職)	川北医学院附属医院　胸外科　（　主治医師　）				
研究先（指導教官）	東京大学大学院　医学系研究科　呼吸器外科　（　中島　淳　教授　）				
研究テーマ	肺移植に関する実験的・臨床的研究 Experimental and clinical research on lung transplantation				
専攻種別	論文博士	□	課程博士	☑	

1．研究概要（1）

1) 目的 (Goal) Ex vivo lung perfusion (EVLP) is reportedly a useful strategy that permits initially rejected donor lungs to be evaluated and reconditioned for successful lung transplantation (LTx). This systematic review and meta-analysis was performed to evaluate the outcomes of EVLP conducted for marginal donor lungs.

2) 戦略 (Approach) We searched PubMed, the Cochrane Library, and EMBASE to select studies describing the results of LTx following EVLP for initially rejected donor lungs compared with standard LTx without EVLP.

3) 材料と方法 (Materials and methods) We performed a meta-analysis to examine donor baseline characteristics, recipient baseline characteristics, and postoperative outcomes.

4) 実験結果 (Results) Of 1380 studies, 9 studies involving 1246 patients met the inclusion criteria. Compared with the non-EVLP group (standard LTx without EVLP), the EVLP group (EVLP of marginal donors following LTx) had similar donor age and sex and recipient baseline age, sex, body mass index (BMI), bridge by ventilator/extracorporeal life support (ECLS)/extracorporeal membrane oxygenation (ECMO), and rate of double LTx but more abnormal donor lung X-rays (P=0.0002), a higher smoking history rate (P=0.03), and worse donor PaO2/FiO2 (P<0.00001). However, there were no significant differences in outcomes between the EVLP and non-EVLP groups with respect to the length of postoperative intubation, postoperative ECMO/ECLS use, length of intensive care unit stay, length of hospital stay, 72-h primary graft dysfunction of grade >3, 30-day survival, or 1-year survival (all P>0.05).

5) 考察 (Discussion) Post-transplant outcomes were similar between EVLP-treated LTx and standard LTx without EVLP, although the quality of donor lungs was worse with EVLP-treated LTx.

6) 参考文献 (References) 1. Cooper JD, Pearson FG, Patterson GA, et al. Technique of successful lung transplantation in humans. J Thorac Cardiovasc Surg. 1987;93:173-181.
2. Klein AS, Messersmith EE, Ratner LE, Kochik R, Baliga PK, Ojo AO. Organ donation and utilization in the United States, 1999-2008. Am J Transplant. 2010;10:973-986.
3. Wallinder A, Ricksten SE, Silverborn M, et al. Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case-control study. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery. 2014;45:40-44; discussion 44-45.
4. Valapour M, Paulson K, Smith JM, et al. OPTN/SRTR 2011 Annual Data Report: Lung. American Journal of Transplantation. 2013;13:149-177.
5. Keshavjee SH, Yamazaki F, Cardoso PF, McRitchie DI, Patterson GA, Cooper JD. A method for safe twelve-hour pulmonary preservation. J Thorac Cardiovasc Surg. 1989;98:529-534.

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title	Tumour size: an unneglectable prognostic factor for patients with thymoma. (Accept)				
掲載誌名 Published journal	European Journal of Cardio-Thoracic Surgery. (IF:3.504)				
	年 月	巻（号）	頁 ～ 頁	言語 Language	English
第1著者名 First author	Dong Tian	第2著者名 Second author	Heng Huang	第3著者名 Third author	Kai-Yuan Jiang
その他著者名 Other authors	Long-Qi Chen				
論文名 2 Title	Outcomes in Lung Transplantation of Marginal Donor after Ex vivo Lung Perfusion: A Systematic Review and Meta-analysis. (Accept)				
掲載誌名 Published journal	J Thorac Cardiovasc Surg. (IF:4.88)				
	年 月	巻（号）	頁 ～ 頁	言語 Language	English
第1著者名 First author	Dong Tian	第2著者名 Second author	Yu Wang	第3著者名 Third author	Haruhiko Shiiya
その他著者名 Other authors	Chang-Bo Sun, Yukari Uemura, Masaaki Sato, Jun Nakajima				
論文名 3 Title	Neoadjuvant Chemotherapy with Irinotecan and Nedaplatin in Single Cycle Followed by Esophagectomy Versus Surgery Alone on cT4 Potential Resectable Esophageal Squamous Cell Carcinoma: A Prospective Nonrandomized Trial for Short-term Outcomes.				
掲載誌名 Published journal	Dis Esophagus. (IF: 2.702)				
	2019 年 3 月	32(3) 巻（号）	頁 ～ 頁	言語 Language	English
第1著者名 First author	Dong Tian	第2著者名 Second author	Lin Zhang	第3著者名 Third author	Yu Wang
その他著者名 Other authors	Liang Chen. Ke-Ping Zhang, Yu-Zhou, Hong-Ying Wen, Mao-Yong Fu				
論文名 4 Title	Experience from AATS Foundation for Thoracic Surgery Training Fellowship: Lung Transplantation in Toronto General Hospital.				
掲載誌名 Published journal	J Thorac Cardiovasc Surg. (IF:4.88)				
	2018 年 8 月	156(2) 巻（号）	929 頁 ～ 930 頁	言語 Language	English
第1著者名 First author	Dong Tian	第2著者名 Second author	Shaf Keshavjee	第3著者名 Third author	
その他著者名 Other authors					
論文名 5 Title					
掲載誌名 Published journal					
	年 月	巻（号）	頁 ～ 頁	言語 Language	
第1著者名 First author		第2著者名 Second author		第3著者名 Third author	
その他著者名 Other authors					

3. 学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載してください

※Describe your presentation as the principal presenter in major academic meetings including general meetings or i

学会名 Conference	27th Annual meeting of Asian Society for Cardiovascular and Thoracic Surgery
演 題 Topic	Depth of Circular and Longitudinal Muscle Invasion in T2 Esophageal Squamous Cell Carcinoma Does Not Affect the Prognosis or Lymph Node Metastasis
開催日 date	2019 年 2 月 21 日 ／ 開催地 venue Chennai, India
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poste ／ 言語 Language ☐ 日本語 ☑ 英語 ☐ 中国語
共同演者名 Co-presenter	Heng, Huang, Kai-Yuan Jiang, Huan-Yu Tang
学会名 Conference	27th Annual meeting of Asian Society for Cardiovascular and Thoracic Surgery
演 題 Topic	Risk factors of lymph node metastasis in T1 esophageal squamous cell carcinoma
開催日 date	2019 年 2 月 21 日 ／ 開催地 venue Chennai, India
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poste ／ 言語 Language ☐ 日本語 ☑ 英語 ☐ 中国語
共同演者名 Co-presenter	Heng, Huang, Kai-Yuan Jiang, Huan-Yu Tang
学会名 Conference	27th Annual meeting of Asian Society for Cardiovascular and Thoracic Surgery
演 題 Topic	10-year Changes in Dietary Habits and Medical Knowledge of Esophageal Cancer in A High-incidence Area
開催日 date	2019 年 2 月 21 日 ／ 開催地 venue Chennai, India
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poste ／ 言語 Language ☐ 日本語 ☑ 英語 ☐ 中国語
共同演者名 Co-presenter	Heng, Huang, Kai-Yuan Jiang, Huan-Yu Tang
学会名 Conference	中华医学会第十八次全国胸心血管外科学术会议暨2018国际胸心血管外科冬季研讨论坛
演 題 Topic	食管癌高发区农村居民饮食和认知的十年变化
開催日 date	2018 年 10 11 日 ／ 開催地 venue 沈阳
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poste ／ 言語 Language ☐ 日本語 ☑ 英語 ☐ 中国語
共同演者名 Co-presenter	黄桁，姜凯元，邓静雅

4. 受賞（研究業績）Award (Research achievement)

名 称 Award name	AATS Graham Foundation for Thoracic Surgery Training Fellow			
	国名 Country name	USA	受賞年 Year of	2018 年 5 月
名 称 Award name				
	国名 Country name		受賞年 Year of	年 月

5．本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	□ 有 ■ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円
受給実績 Receipt record	□ 有 ■ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

6．他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	■ 有 □ 無
助成機関名称 Funding agency	Chinese Government
奨学金名称 Scholarship name	China Scholarship Council
受給期間 Supported period	2018 年 9 月 ～ 2021 年 8 月
受給額 Amount received	15万円/月 円

7．研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。 Attach a copy of the article described below

報道発表 Press release	□ 有 ■ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞 ・雑誌 ・Web site ・記者発表 ・その他（ ）		
発表タイトル Released title			

8．本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有 ■ 無	出願国 Application	
出願内容（概要） Application contents			

9．その他 Others

指導責任者（署名） 中島 淳

日中笹川医学奨学金制度(学位取得コース)中間評価書
課程博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　研究者番号：G4004　作成日　：2019 年 3 月13日

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研究先（指導教官）	東京大学大学院　医学系研究科　呼吸器外科学（　中島　淳　教授）				
研究テーマ	肺移植に関する実験的・臨床的研究 Experimental or clinical research on lung transplantation				
専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	優 学業成績係数=	取得単位数 取得単位数＝８／取得すべき単位総数＝３２
学生本人が行った研究の概要	（1）　臨床肺移植においてドナー肺の不足が問題となる。ドナー肺の機能が採取時に不十分な場合、欧米では"Ex vivo lung perfusion (EVLP)"を行うことがある。すなわち体外に取り出された肺の動脈から保存液を持続的に注入、肺静脈から排出させ再び動脈に灌流、同時に気管支から換気を行う装置に肺を置き、薬剤などを用いて体外で肺を治療するものである。全世界での報告例をもとにメタアナリシスを行った（本人の研究報告書の内容） （2）　その他、ラットによる同種片肺移植実験に従事し、今年度は肺移植手術モデルの確立および長期生存を達成させた。 （3）	
総合評価	【良かった点】 2018 年 4 月入学後に上記(1)を含め 4 編の英文論文を出版または掲載決定させた。また、次年度を目指しラット肺移植モデルを確立させた。 【改善すべき点】 改善すべき点というわけではないが、大学院学生期間中にメインテーマであるラット肺移植モデルを用いた拒絶反応、特に慢性拒絶反応の機序解明のための研究を来年度は本格的に行ってもらうことを期待している。 【今後の展望】 ラット肺移植モデルを用いた拒絶反応、特に慢性拒絶反応の機序解明のための研究をさらに発展させる。	
学位取得見込	大学院生の期間中(4 年間もしくはそれ以内)に取得する見込みである。	
評価者（指導教官名）	中島 淳 ㊞	

Diseases of the Esophagus (2018) **0**, 1–11
DOI: 10.1093/dote/doy080

DISEASES OF THE ESOPHAGUS

ISDE The International Society for Diseases of the Esophagus

Original Article

Neoadjuvant chemotherapy with irinotecan and nedaplatin in a single cycle followed by esophagectomy on cT4 resectable esophageal squamous cell carcinoma: a prospective nonrandomized trial for short-term outcomes

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SUMMARY. **Neoadjuvant chemotherapy (NAC) significantly extends survival in advanced esophageal squamous cell carcinoma (ESCC), but the short-term outcomes for cT4 ESCC remain controversial. Many NAC regimens have been previously reported, although no study has reported a regimen of irinotecan and nedaplatin for cT4 potential resectable ESCC. We evaluated the short-term outcomes of NAC with irinotecan and nedaplatin in a single cycle followed by esophagectomy on cT4 resectable ESCC. A total of 51 patients with cT4 potentially resectable ESCC were eligible for this study. Twenty of these patients underwent NAC, and the other 31 patients underwent surgery alone. The toxicities and response of NAC were evaluated. The clinicopathologic characteristics, responses, toxicities, surgical outcomes, postoperative complications, and survival time between the two groups were analyzed. No significant differences were found in clinicopathologic characteristics between the groups ($P > 0.05$). The response rate of NAC was 75% (15/20). The differences in the long-axis diameter of the tumor and cT stage between pre- and post-NAC were significant ($P < 0.05$). Twenty-four toxic events occurred in 11 patients of the NAC group, and 20/24 of these were mild. The R0 resection rates in the NAC group and the surgery alone group were 85% and 64.5%, with no statistically significant difference ($P > 0.05$). Differences in the pathological T stage and pathological tumor-node-metastasis (TNM) stage were significant ($P < 0.05$). The overall survival (OS) time and mortality in the NAC group versus the surgery alone group were 31.57 ± 3.06 months versus 15.24 ± 1.46 months and 25% versus 61.3%, respectively. The differences in OS and mortality were significant ($P < 0.05$). The NAC group and R0 resection were significant and independent predictors of positive prognosis. NAC with irinotecan and nedaplatin in a single cycle followed by esophagectomy on cT4 resectable ESCC as a new NAC is safe and effective.**

KEY WORDS: **esophageal squamous cell carcinoma, irinotecan, nedaplatin, neoadjuvant chemotherapy, short-term outcome.**

INTRODUCTION

Esophageal cancer penetrates the esophageal wall and easily involves adjacent organs because no tunicae serosa is present.[1] Patients are usually diagnosed at an advanced stage due to this particular feature. An esophageal cancer involving adjacent structures (aorta, major airway, lung, diaphragm, pulmonary vein, pleural, and pericardium) is defined as T4 disease, which results in a poor R0 resection rate and survival time.[2-4]

Surgery alone may be performed in T4 patients, although its prognostic benefit and R0 resection rate remain dismal.[5-11] Matsubara *et al.*[12] concluded that patients with macroscopic-T4 but not pathologic-T4 tumors had favorable outcomes and that only patients with definitive evidence of unresectability should be excluded from esophagectomy. In addition, Tachibana *et al.*[13] and Chen *et al.*[14] demonstrated that esophagectomy of cT4 can achieve the best improvement in swallowing and the longest survival with an

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Consent for publication: Informed consent for publishing the individual patient data was obtained from all of the participants.
Specific author contributions: Manuscript preparation: Dong Tian, Lin Zhang, Yu Wang; Reference literature review: Dong Tian, Lin Zhang, Yu Wang; Manuscript writing: Dong Tian, Lin Zhang, Yu Wang; Clinical data collection: Liang Chen, Keping Zhang, Yu Zhou, Hongying Wen; Program design, manuscript amendment: Maoyong Fu.
All the authors read and approved the final manuscript.

acceptable mortality rate. The optimal management for patients with potentially resectable cT4 esophageal squamous cell carcinoma (ESCC) remains unknown.

The overall survival (OS) time of cT4 ESCC has been improved by the development of multidisciplinary treatments, as reported in recent studies.[15-19] Many neoadjuvant chemotherapy (NAC) regimens have been reported in previous studies but were associated with different prognostic outcomes.[20-29]

Irinotecan has been administered as NAC to advanced esophageal cancer patients in previous trials. However, most of these treated cases were esophageal adenocarcinoma with a low pathologic complete response (pCR) rate. A response to this chemotherapy was found in ESCC, although the number of cases was limited.[30,31] We thus considered that the NAC regimen with irinotecan and nedaplatin would also be applicable in NAC regimens. Additionally, the short-term outcomes and treatment toxicities for cT4 ESCC remain controversial.

Most NAC requires two cycles of preoperative chemotherapy, but a single cycle has also conferred positive responses in some cases. Tumors rapidly develop resistance to chemotherapy, and responses are generally short lived.[32] Karagiannis *et al.*[33] showed that some NAC increases the risk of metastatic dissemination through a tumor microenvironment of metastasis (TMEM)-mediated mechanism, despite decreasing the tumor size. This may be due to drug resistance in NAC with lower doses or longer preoperative time intervals. Recently, Fujiwara *et al.*[34] compared the perioperative results and prognoses of patients who underwent complete (two cycles) or incomplete (single cycle) NAC because of adverse events or the patient's refusal of treatment. They found perioperative outcomes and long-term prognosis of patients with locally advanced ESCC were not significantly influenced, even if the patients did not receive a complete cycle of NAC. For these reasons, we hypothesized that one cycle of NAC as a pulse therapy may prevent drug resistance and the risk of metastatic dissemination. This one-cycle treatment may achieve a comparable effect to two or more NAC cycles because a similar total dose is used. If downstaging can be achieved by a single cycle, chemotherapy toxicities and relapse can be avoided.

In this study, we evaluated the short-term outcomes of a single cycle of irinotecan and cisplatin NAC followed by esophagectomy in cT4 potentially resectable ESCC patients.

PATIENTS AND METHODS

Patients

Between January 2014 and March 2017, 970 consecutive patients who were histologically diagnosed with ESCC and planned to undergo surgery presented at the Affiliated Hospital of North Sichuan Medical College. In total, 109 patients were defined as cT4 according to the TNM classification of the American Joint Committee on Cancer (AJCC) & The Union for International Cancer Control (UICC).[35] Of these 109 patients, 51 entered this prospective trial.

The criteria for inclusion of patients in this prospective trial were as follows: (1) ESCC in the thoracic esophagus, (2) cT4 according to the AJCC & UICC 8th edition classification, (3) expected survival time greater than 3 months, (4) general condition adequate to tolerate single-cycle NAC and/or esophagectomy, (5) evaluated as resectable esophageal cancer by pretreatment examinations, and (6) provided written informed consent.

Exclusion criteria were as follows: (1) received other treatment (chemotherapy/radiotherapy/palliative therapy) that may affect the prognosis or additional cycles of perioperative chemotherapy, (2) distant metastasis, (3) esophageal adenocarcinoma and esophagogastric junction carcinoma, (4) any concurrent primary cancer at other organs, (5) rejected operative surgery after NAC, (6) esophageal perforation or tracheoesophageal fistula, and (7) >80 years old.

The staging evaluation before treatment included the following procedures: (1) necessary general physical examination, (2) esophagogastroduodenoscopy (EGD) and biopsy, (3) contrast esophagography, (4) cervical and abdominal ultrasonography (US) and endoscopic ultrasonography (EUS), (5) contrast-enhanced computed tomography (CT) of the neck, chest, and upper abdomen, (6) bone scintigraphy, and 7) bronchoscopy performed only for the cancer in the upper or middle thoracic esophagus.

All patients were staged according to the AJCC & UICC criteria. cT4 was defined using contrast-enhanced CT, contrast esophagography, and bronchoscopy (upper or middle thoracic ESCC), and EUS. Lymphatic metastasis was assessed by morphology using cervical and abdominal US, EUS, and contrast-enhanced CT. Distant metastasis was determined using contrast-enhanced CT and bone scintigraphy. Esophageal cancer without distant metastasis invading the pleura, pericardium, diaphragm, and fat plane in the triangular space among the esophagus, aorta, and spine could be defined as a resectable cT4 tumor. However, if the aorta, trachea, and spine were invaded by esophageal cancer, surgery could not be performed.[35]

The excluded 58 patients included 20 with distant organ metastases, 14 who underwent previous chemotherapy and/or radiotherapy, 8 with a low performance status index for surgery or chemotherapy, 7 who were >80 years old, and 9 who refused esophagectomy after an active response to NAC.

This study was conducted with the approval of the Ethics Committee of the Affiliated Hospital of North Sichuan Medical College.

METHODS

Chemotherapy regimens

This was a prospective, nonrandomized trial that included patients who made an informed decision regarding whether to receive NAC followed by esophagectomy or esophagectomy alone. When patients decided to receive NAC followed by esophagectomy, they underwent the single cycle of NAC first. The chemotherapy regimen consisted of 120 mg/m^2 irinotecan (Jiangsu Hengrui Medicine Co., Ltd., Lianyungang, Jiangsu, P. R. China) on day 1, day 8, and day 15 for 3 hours as a drip intravenous infusion and 20 mg/m^2 nedaplatin (Qilu Pharmaceutical, Jinan, Shandong, P. R. China) administered intravenously from day 1 to day 5 for 1 hour combined with antiemetic and antimyelosuppression to alleviate toxicities. Then, patients in the NAC group received a subcutaneous injection of 6 mg PEG-rhG-CSF (Qilu Pharmaceutical, Jinan, Shandong, P. R. China) on day 15 to prevent myelosuppression. If the total cell counts of the bone marrow were <30,000/mm^3, surgery was delayed for 1 week or more. For patients with severe dysphagia, total parenteral nutrition was used, with or without additional oral administration of liquid nutrients. Soft or normal foods were given to patients if dysphagia improved. Examinations for staging evaluation before treatment were performed 4–6 weeks after NAC (before surgery).

Surgery

Esophagectomy was completed in both groups. NAC group patients underwent surgery 4–6 weeks after completing NAC. Before surgery, restaging evaluation was performed again, as performed prechemotherapy. Patients with esophageal cancer in the upper third of the thoracic esophagus underwent McKeown esophagectomy and were treated with three-field lymphadenectomy. The type of esophagectomy (Sweet, Ivor-Lewis, or McKeown esophagectomy) performed on middle third and lower third of the thoracic esophagus was at the discretion of the surgeon.

Clinical indexes

The database was queried to include all patients and the following variables: clinicopathologic characteristics, NAC-associated toxicities, postoperative complications, R0 resection, CR + PR and OS time. Survival time for all of the patients was calculated from the start of initial treatment until death from any cause or the final follow-up visit.

Evaluation of residual tumor (R) was classified as follows: R0, no residual tumor; R1, suspicion of residual tumor or microscopic residual tumor; and R2, macroscopic residual tumor.[36] Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 4.0 (NCI CTC v 4.0).[37]

Briefly, the responses were classified as follows: complete response (CR), complete disappearance of all clinical evidence of existing lesions during chemotherapy; partial response (PR), a decrease in tumor size of more than 30% during chemotherapy; progressive disease (PD), an increase in tumor size of more than 20% compared with the initial size; and stable disease (SD), any changes in tumor size that could be classified as neither a PR nor PD. Patients with a tumor showing a CR or PR were defined as major responders, and those with a tumor showing SD or PD were defined as nonresponders.[38]

Follow-up

Patients were followed up by monthly home visits or telephone interviews to determine their living conditions and to confirm they were alive. All of the patients were followed up until May 2017 or death. Patients were closely observed by general physical examination, contrast esophagography, cervical and abdominal US and EUS, contrast-enhanced CT of the cervical, chest and upper abdomen, and bone scintigraphy every 3 months and by EGD every 6 months after the surgery.

Statistical analysis

Follow-up data after treatment were available for all patients. Statistical analyses were performed with SPSS 22.0 (SPSS, Inc., Chicago, IL). Data were reported as the frequencies, means, and medians with percentages. The Chi-square test was used for comparison of the categorical variables. OS curves were plotted by the Kaplan-Meier method. Log-rank tests were applied to identify significant differences in survival among groups. We used the Cox proportional hazards model for multivariable OS analysis. Variables potentially related to the risk of OS with $P < 0.10$ on univariate analysis were included in the multivariate analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Clinicopathologic characteristics

Between January 2014 and March 2017, 51 patients were enrolled in this study. Twenty patients chose NAC with irinotecan and cisplatin in a single cycle followed by esophagectomy, and the other 31 patients

Table 1 Summary of patient clinicopathologic characteristics

Parameters	All patients ($N = 51$)	NAC ($N = 20$)	Surgery alone ($N = 31$)	P
Age (mean ± SD) year (range)	61.2 ± 6.57 (43–76)	60.3 ± 7.18 (43–69)	61.8 ± 6.20 (49–76)	0.44*
Gender				0.98**
Male	42(82.4%)	17(85%)	25(80.6%)	
Female	9(17.6%)	3(15%)	6(19.4%)	
Tumor localization				0.35**
Upper third	4(7.8%)	1(5%)	3(9.7%)	
Middle third	32(62.8%)	15(75%)	17(54.8%)	
Lower third	15(29.4%)	4(20%)	11(35.5%)	
Initial long-axis diameter of tumor (mean ± SD) cm (range)	5.3 ± 0.89 (3.5–8.0)	5.3 ± 0.95 (4–8)	5.2 ± 0.86 (3.5–7)	0.90*
BMI (mean ± SD) (range)	24.2 ± 6.57 (19.5–31.2)	24.2 ± 2.78 (19.5–31.2)		0.95*
Clinical N stage				0.33**
cN0	23(45.1%)	8(40.0%)	15(48.4%)	
cN1	17(33.3%)	9(45%)	8(25.8%)	
cN2	11(21.6%)	3(15%)	8(25.8%)	
cT4 invaded organs				0.66**
fat plane in triangular space†	17(33.3%)	6(30%)	11(35.5%)	
pleura	22(43.2%)	9(45%)	13(41.9%)	
pericardium	8(15.7%)	3(15%)	5(16.1%)	
diaphragm	4(7.8%)	2(10%)	2(6.5%)	

*Student's t test was used; **$\chi 2$ test or Fisher's exact test was used.
†The fat plane in the triangular space between the esophagus, aorta, and spine was obliterated.
BMI, body mass index; NAC, neoadjuvant chemotherapy.

underwent surgery alone. All NAC group patients completed the single-cycle regimen. All 51 patients had locally advanced potentially resectable cT4 SCC and underwent esophagectomy. Of the 51 patients, 42 (82.4%) were male, and 9 (17.6%) were female. The median patient age was 61.2 ± 6.57 years (range: 43–76 years). The tumor location of all patients was 4 (7.8%), 32 (62.8%), and 15 (29.4%) in the upper, middle, and lower third, respectively. The average length of the tumor before treatment was 5.25 ± 0.89 cm (range: 3.5–8.0 cm). The average body mass index (BMI) was 24.15 ± 2.68 (range: 19.49–31.22). No significant differences in gender distribution, age, tumor location, initial long-axis diameter of the tumor, and clinical N stage before treatment were observed between the NAC group and the surgery alone group ($P = 0.30$, $P = 0.46$, $P = 0.44$, $P = 0.44$, and $P = 0.09$, respectively) (Table 1).

Response to NAC

All of the NAC group patients underwent NAC with a single cycle of irinotecan and nedaplatin. Four to six weeks after the NAC regimen, there were 4 (20%) patients with CR, 11 (55%) patients with PR, and 5 (25%) patients with SD. None of the patients encountered PD. Accordingly, the response rate to the single-cycle NAC regimen was 75% (15/20). The long-axis diameter of the tumor before NAC was 5.3 ± 0.95 cm and 2.85 ± 2.05 cm 4–6 weeks after NAC. The differences in the long-axis diameter of the tumor and in the cT stage between pre-NAC and post-NAC were significant ($P < 0.05$). However, there was no significant difference in cN stage between pre-NAC and post-NAC ($P > 0.05$) (Table 2).

Toxicity

NAC with irinotecan and cisplatin in a single cycle was generally well tolerated. The overall toxicities experienced by the patients during chemotherapy are listed in Table 3. Twenty-four toxic events occurred in 11 patients (55%) of the NAC group and 20/24 of these were mild (grade 1–2). The toxic rates of leukopenia, nausea and vomiting, diarrhea, alopecia, and renal dysfunction were 35%, 30%, 40%, 5%, and 10%, respectively. The major toxicities were hematologic (leukopenia and neutropenia) and gastrointestinal reaction (nausea or/and vomiting, diarrhea), with 2 (10%) of the patients experiencing grade 3 or 4 leukopenia and neutropenia and 2 (10%) patients with grade 3 gastrointestinal reactions. All of the toxicities were within expectations and were manageable, and no treatment-related death occurred. No patient canceled their operation due to NAC toxicity.

Surgical outcome

As shown in Table 4, more than half of the patients in the NAC and surgery alone groups received the Ivor-Lewis or McKeown procedure (55% and 54.8%, respectively). Seventeen of the 20 patients (85%) in the NAC group received an R0 resection compared with 20 of the 31 patients (64.5%) in the surgery alone

Table 2 Response to NAC with irinotecan and nedaplatin in a single cycle

	Pre-NAC	Post-NAC	*P*
Long-axis diameter of tumor (mean ± SD) cm	(5.27 ± 0.95)	(2.85 ± 2.05)	0.00*
cT stage(%)†			0.00**
cT4	20(100%)	5(25%)	
Others	0(0%)	15(75%)	
cN stage(%)†			0.79**
cN0	8(40%)	10(50%)	
cN1	9(45%)	8(40%)	
cN2	3(15%)	2(10%)	

*Student's t test was used; ** $\chi 2$ test or Fisher's exact test was used.
†8th edition of the AJCC & UICC.
cN stage, clinical N stage; cT stage, clinical T stage; NAC, neoadjuvant chemotherapy.

Table 3 Toxicities experienced by the patients during NAC

	NCICTC version 4.0 common toxicity criteria					
	Grade 1	Grade 2	Grade 3	Grade 4	All grades (%)	Grade 3/4 (%)
Leukopenia	3	2	1	1	7(35%)	2(10%)
Nausea or vomiting	3	3	0	0	6(30%)	0(0%)
diarrhea	3	3	2	0	8(40%)	2(10%)
Alopecia	1	0	0	0	1(5%)	0(0%)
Renal dysfunction	1	1	0	0	2(10%)	0(0%)

NAC, neoadjuvant chemotherapy; NCICTC version 4.0, National Cancer Institute Common Toxicity Criteria, version 4.0.

group, and 50% and 48.4% of the patients had lymph node metastasis in the NAC group and the surgery alone group, respectively. The mean resected lymph nodes and metastatic lymph nodes in the NAC group versus the surgery alone group were 16.75 ± 3.63 versus 16.75 ± 3.63 and 1.55 ± 2.14 versus 1.55 ± 2.14, respectively. There were no significant differences in the type of esophagectomy, surgical radicality (R0 vs. R1 + R2), the nature of the lymph nodes, mean resected lymph nodes, mean metastatic lymph node, pathological N stage, or histopathological grading between the NAC group and surgery alone group ($P = 0.99$, $P = 0.20$, $P = 0.91$, $P = 0.12$, $P = 0.68$, $P = 0.88$ and $P = 0.99$, respectively). In addition, The R0 and R1 patients in the NAC group versus surgery group were 17 versus 20 and 2 versus 7, respectively. There was also no significant difference ($P = 0.27$) between the NAC group and the surgery group. However, the differences in pathological T stage and TNM stage between the NAC group and the surgery alone were significant ($P = 0.00$ and $P = 0.001$, respectively).

Postoperative complications

Nine postoperative events occurred in 5 patients (25%) of the NAC group, and 18 postoperative events occurred in 11 patients (35.5%) of the surgery alone group. The main complications in the two groups were infection complications (pyothorax, pneumonia, and surgical site infection), surgery-related complications (chylothorax, anastomotic leak, and recurrent nerve paralysis) and other complications (deep venous thrombosis and hypoproteinemia). There was no operative mortality patient in either group (postoperative within 30 days) but 1 (3.2%) hospital mortality (2 months after operation) patient in the surgery alone group. The patient died of anastomotic leak and pyothorax due to palliative resection (R2 resection). Regarding postoperative complications, the incidences of infection complications, surgery-related complications, and other complications for the NAC group versus the surgery alone group were 3 (15%) versus 7 (22.6%), 2 (10%) versus 5 (16.1%), and 4 (20%) versus 6 (19.4%), respectively. No differences were observed between the NAC and surgery alone groups (Table 5).

Short-term survival outcomes

With a median follow-up of 15.06 ± 8.52 months (range: 2–38 months), 24 (47.1%) out of 51 patients died of disease progression or postoperative complications, 5 (25%) in the NAC group, and 19 (61.3%) in the surgery alone group. To evaluate whether the NAC group and surgery alone group had different outcomes, the survival rates were compared. The median OS for all 51 patients was 22.89 ± 2.16 months. The OS was 31.57 ± 3.06 months in the NAC group versus 15.24 ± 1.46 months in the surgery alone group. The differences in mortality and OS between the NAC group and the surgery alone group were significant ($P = 0.01$ and $P = 0.001$, respectively). The Kaplan-Meier curves are shown in Figure 1.

Table 4 Surgical outcome in the NAC group and surgery alone group

	NAC ($N = 20$)	Surgery alone ($N = 31$)	P
Type of esophagectomy			0.99*
Sweet (left chest)	9 (45%)	14 (45.2%)	
Ivor-Leiws or McKeown(right chest)	11 (55%)	17 (54.8%)	
Surgical radicality			0.11**
R0	17 (85%)	20 (64.5%)	
R1 + R2	3 (15%)†	11 (35.5%)‡	
Lymph node			0.91*
Node-negative cases(%)	10 (50%)	15 (48.4%)	
Node-positive cases(%)	10 (50%)	16 (51.6%)	
Mean resected lymph node	16.75 ± 3.63	14.32 ± 6.20	0.12**
Mean metastasis lymph node	1.55 ± 2.14	1.84 ± 2.58	0.68**
Pathological T stage§,¶			0.00*
pT0	4 (20%)	0 (0%)	
pT1	2 (10%)	0 (0%)	
pT2	7 (35%)	0 (0%)	
pT3	4 (20%)	6 (19.4%)	
pT4	3 (15%)††	25 (80.6%)‡‡	
Pathological N stage§,§§			0.88*
pN0	10 (50%)	15 (48.4%)	
pN1	6 (30%)	7 (22.6%)	
pN2	3 (15%)	7 (22.6%)	
pN3	1 (5%)	2 (6.4%)	
Pathological differentiation§			0.99*
Well differentiated	5 (25%)	8 (25.8%)	
Moderately differentiated	12 (60%)	18 (58.1%)	
Poorly differentiated	3 (15%)	5 (16.1%)	
Pathological stage§,¶¶			0.001*
pStage I	8 (40%)	0 (0%)	
pStage II	1 (5%)	1 (3.2%)	
pStage III	9 (45%)	17 (54.9%)	
pStage IV	2 (10%)	13 (41.9%)	

*$\chi 2$ test or Fisher's exact test was used; **Student's t test was used.
†Three patients were performed as R1/R2 resection due to pleura (1), pericardium (1), and diaphragm (1) invading and residual tumor existing, respectively; ‡Eleven patients were performed as R1/R2 resection due to aorta (6) and pleura (5) invading and residual tumor existing; §8th edition of the AJCC&UICC; ¶ypT for NAC group; ††Three patients were diagnosed as ypT4 due to pleura (1), pericardium (1) and diaphragm (1) invading, respectively; ‡‡patients were diagnosed as pT4 due to aorta (6), the fat plane in the triangular space among the esophagus, aorta and spine (7), pleura (10), pericardium (1) and diaphragm (1) invading, respectively; §§ypN for NAC group; ¶¶yp stage for NAC group.
NAC, neoadjuvant chemotherapy; R0, no residual tumor; R1, suspicion of residual tumor or microscopic residual tumor; R2, macroscopic residual tumor.

Variables predicting short-term survival

According to the univariate analysis, treatment group ($P = 0.01$) and surgical radicality ($P = 0.00$) were significant prognostic factors. No significant differences in age, gender, long-axis diameter of the tumor, type of esophagectomy, postoperative complications, lymph node resection, lymph node metastasis, and BMI were observed ($P = 0.23$, $P = 0.57$, $P = 0.75$, $P = 0.51$, $P = 0.37$, $P = 0.36$, $P = 0.12$ and $P = 0.78$, respectively). The multivariate analysis also revealed that treatment group ($P = 0.007$) and surgical radicality ($P = 0.01$) were significant prognostic factors. There were no significant differences in age, gender, long-axis diameter of the tumor, type of esophagectomy, postoperative complications, lymph node resection, lymph node metastasis, and BMI ($P = 0.13$, $P = 0.10$, $P = 0.12$, $P = 0.06$, $P = 0.10$, $P = 0.73$, $P = 0.69$ and $P = 0.53$, respectively) (Table 6).

DISCUSSION

Esophageal cancer is one of the most aggressive and common cancers with a low 5-year survival rate after curative surgery.[39] To improve outcomes, current evidence supports the effects of NAC on patients with advanced esophageal cancer.[40] A recent study demonstrated that NAC improved R0 resection and OS compared with surgery alone, with a 12% decrease in the mortality hazard.[41] However, the optimal chemotherapy regimen for advanced esophageal cancer is also uncertain. Additionally, the results of the JCOG 9907[20] study aided in the approval of NAC with FP as a standard regimen in Japan. However, the response rate remained unsatisfactory at 38%.

One or more cycles of NAC were used in most previous studies with the expectation of more responders.[34,42] However, there were no significant differences in 5-year OS rate and median survival times

Table 5 Postoperative complications in the NAC group and surgery alone group

	NAC group ($n = 20$)	Surgery alone group ($n = 31$)	P^*
Total complications	5(25%)	11(35.5%)	0.43
Hospital mortality (> 30 days)	0	1(3.2%)	1.00
Infection complications	3(15%)	7(22.6%)	0.76
Pyothorax	0(0%)	2(6.5%)	
Pneumonia	2(10%)	3(9.7%)	
Surgical site infection	1(5%)	2(6.5%)	
Surgical-related complications	2(10%)	5(16.1%)	0.84
Chylothorax	0(0%)	1(3.2%)	
Anastomotic leak	1(5%)	2(6.5%)	
Recurrent nerve paralysis	1(5%)	2(6.5%)	
Other complications	4(20%)	6(19.4%)	1.00
DVT	1(5%)	2(6.5%)	
Hypoproteinemia	3(25%)	4(12.9%)	

*χ^2 test or Fisher's exact test was used.
DVT, deep venous thrombosis; NAC, neoadjuvant chemotherapy.

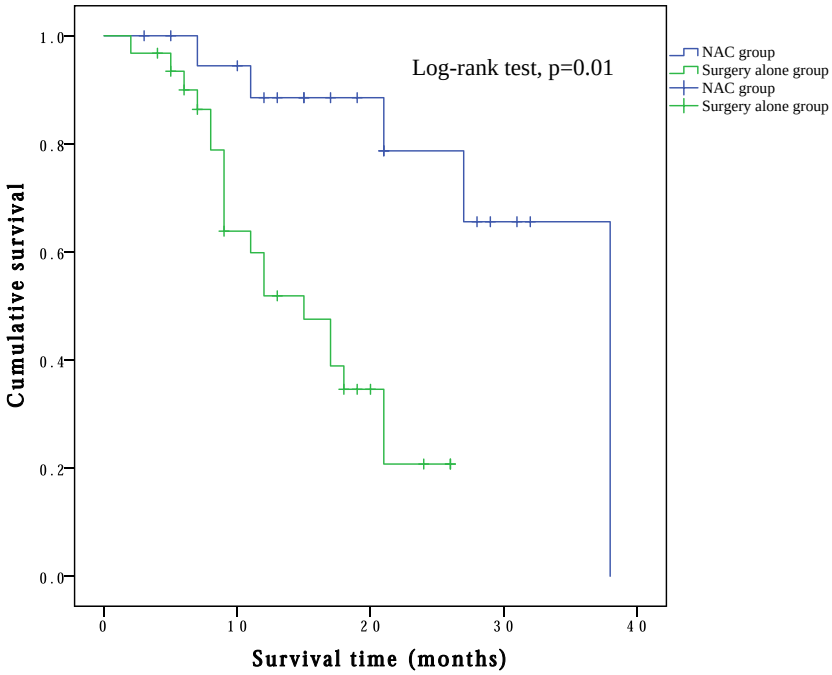

Fig. 1 Kaplan-Meier curves of NAC group and surgery alone group on survival outcomes. The mean overall survival period in NAC group and surgery alone group were (31.57 ± 3.06) months and (15.24 ± 1.46) months, respectively. The differences of OS between two groups were significant ($P < 0.05$)

between early and late responders.[15,42] Therefore, considering the waste of hospital costs and resources and the decreased toxicities, we used a single cycle NAC. In addition, tumors did not further progress during this shorter period.

Irinotecan (CPT-11, Camptosar), a semisynthetic camptothecin, is an inhibitor of the enzyme topoisomerase I. Irinotecan has emerged as a significant new chemotherapeutic agent with a broad spectrum of antitumor activity, including effectiveness against esophageal and gastric cancer.[30,43] Recently, irinotecan has often been preferred in advanced esophageal cancer as a preoperative chemoradiotherapy. Most of these cases were esophageal adenocarcinoma with a low pCR rate. A positive response was found in a limited number of ESCC cases in a previous trial.[44] Irinotecan plus a platinum-based chemotherapy regimen in advanced esophageal cancer corresponded to a total response rate of 57% (ESCC 66%), including a 6% complete response rate.[27] Nedaplatin (cis-diamine-glycolate platinum, CDGP) is a less nephrotoxic analog of CDDP, a second-generation platinum derivative that has shown potent antitumor activity against lung, testicular, esophageal,

Table 6 Univariate and multivariate analysis of prognostic factors according to OS

Prognostic factors	Univariate analysis		Multivariate analysis	
	HR(95%CI)	*P* value	HR(95%CI)	*P* value
Groups (NAC/Surgery alone)	0.211(0.061–0.730)	0.011	7.853(1.747–35.305)	0.007
Age (<60/≥60)	2.010(0.635–6.359)	0.232	0.445(0.155–1.274)	0.13
Gender (male/female)	0.661(0.155–2.813)	0.574	0.371(0.113–1.220)	0.10
Long-axis diameter of tumor (<5/≥5)	0.824(0.251–2.706)	0.749	0.396(0.123–1.277)	0.12
Type of esophagectomy (left/right chest)	1.455(0.480–4.409)	0.507	0.361(0.127–1.024)	0.06
Surgical radicality (R0/R1 + R2)	0.033(0.004–0.280)	0.000	6.157(1.457–26.024)	0.01
Postoperative complications (Yes/No)	0.583(0.177–1.924)	0.374	2.359(0.838–6.641)	0.10
Lymph node resection (<12/≥12)	1.917(0.460–7.831)	0.360	0.821(0.263–2.561)	0.73
Lymph node metastasis (+/−)	0.413(0.134–1.274)	0.121	1.327(0.325–5.412)	0.69
BMI (≤25/>25)	1.176(0.371–3.728)	0.782	0.529(0.742–1.882)	0.53

OS, overall survival; NAC, neoadjuvant chemotherapy; R0, no residual tumor; R1, suspicion of residual tumor or microscopic residual tumor; R2, macroscopic residual tumor.

gynecological, and head and neck cancers. Hydration is unnecessary for nedaplatin treatment.[45] Therefore, our regimen contained irinotecan and nedaplatin as a single cycle NAC with the expectation of better outcomes.

Clinicopathologic characteristics and response

In this study, there was no significant difference in the clinicopathologic characteristics before treatment between the NAC and surgery alone groups. The objective of NAC is to reduce the size of the primary lesion and control lymph node metastasis and micrometastasis to achieve downstaging so that a better outcome can be expected when surgical resection is performed.[46] In this study, the effective response rate was 75%. This rate was slightly higher than that reported in previous studies evaluating irinotecan-based regimens.[27,44] This may be due to the differences in cancer stage and details of the NAC regimen.

Four (20%) patients had T0N0M0 status, which was better than other NAC regimens.[21,28,47] However, there were no significant differences in pathological N and G status ($P > 0.05$). Motoori *et al.*[48] also reported a similar conclusion that there was no change in N stage after NAC. This finding may be partly attributed to the fact that we administered NAC to advanced ESCC patients mainly with clinically node-positive esophageal cancer. However, this protocol differed from other NAC regimens administered to locally advanced esophageal cancer patients with downstaging of either the T or N status.[29,45,49] The different responses between the T stage and the N stage may be due to the drug action mechanism and the characteristics of tumor invasion. The effects of the NAC regimen can also be assessed by the long-axis diameter of the tumor.[50] In this study, there was a significant difference between pre- and post-NAC on long-axis diameter of tumor, which showed the effective response of this NAC regimen ($P < 0.05$). Azria D *et al.*[51] obtained a significantly better prognosis in patients responding to NAC than nonresponders and surgery alone patients.

Toxicity

Although the outcomes of patients who received NAC were favorable, the major toxicities were hematologic (leukopenia and neutropenia), and gastrointestinal reactions (nausea or/and vomiting, diarrhea) and toxicities were major concerns. Frequencies of grade 3/4 leucopenia of 33.3% and of neutropenia of 90% were reported in other studies.[23,25,52–57] In our study, grade 3/4 toxicity developed in 4 (20%) of the 20 patients who underwent the single-cycle NAC. All of the toxicities were manageable, and none of the patients died of NAC-related causes. Our NAC regimen was notably milder than previous regimens, which may be due to the single cycle in our regimen and the prophylactic use of antiemetic and antimyelosuppression to alleviate toxicities.

Surgical outcome and complications

Previous studies demonstrated that NAC did not increase perioperative morbidity and mortality even in minimally invasive esophagectomy (MIE). The Medical Research Council Oesophageal Cancer Working Party reported that the total postoperative complication rates in NAC and surgery alone groups were 41% and 42%, respectively.[24,28,58,59] However, lower postoperative complication rates were observed in our study. Regarding the complication rates, there was no statistically significant difference between the two groups ($P > 0.05$). These results indicate that NAC with irinotecan and nedaplatin administered in a single cycle as a new NAC regimen followed by esophagectomy of cT4 resectable tumors is safe.

The rates of R0 resections in previous studies ranged from 76% to 100%.[60-62] These R0 resection rates were similar to that our study. Our NAC regimen achieved a satisfactory rate of 85% for R0 resection.

Chan *et al.*[63] reported that the R0 resection (curative resection) rate was about 80%, which was similar to that in our trial. Additionally, the Medical Research Council Oesophageal Cancer Working Party reported that the R0 resection rates in NAC and surgery alone groups were similar and did not significantly differ.[64] In our study, although the resection rate in the NAC group was better than that in the surgery alone group (85% vs. 64.5%), the difference between the rates was not significant ($P > 0.05$).

The mean numbers of lymph nodes sampled in the NAC group and the surgery alone group were less than those reported in a previous study.[65] The mean numbers of metastatic lymph nodes in our study had no significant difference between the NAC group and the surgery alone group ($P > 0.05$). Additionally, the number of cases of metastatic lymph nodes was greater than that reported in another study,[64] which reason may be due to different pathological stages.

Short-term survival outcomes

Our study results clearly showed that the NAC group and R0 resection were significant and independent predictors of positive prognosis in both univariate and multivariate analyses. According to the results of mortality and OS, there were better short-term survival outcomes in the NAC group than in the surgery alone group. The differences in mortality and OS between the NAC group and the surgery alone group were significant. Another study also showed a significant OS benefit for patients in the NAC group. The median OS time was 16 months in the NAC group compared with 12 months in the surgery alone group.[22] However, the results of a prospective randomized study in North America comparing NAC followed by surgery versus surgery alone showed no statistically significant differences in the median OS (14.9 months vs. 16.1 months).[65] The reason for these differences is unclear but may be due to different NAC regimens.

Another important prognostic factor was R0 resection, as shown in the univariate and multivariable analyses. Patients in the NAC group who underwent R0 resection demonstrated an improved survival time.[16,66–68] In our study, although the resection rate in the NAC group was better than that in the surgery alone group (85% vs. 64.5%), the difference between these rates was not significant ($P > 0.05$). This may be due to the limited number of cases in our study.

LIMITATIONS

Some inevitable limitations are present in this study. First, the sample size was considered small, although several comparisons reached statistical significance, which could be due to the time limitation and inclusion criteria. Further studies with larger sample sizes may lead to more accurate results. Second, the inclusion of patients from a single center limits the external generalizability of the results. Third, this study was not randomized. A randomized control trial could not be performed for patients with cT4 esophageal cancer because many patients refuse for their treatment to be determined randomly and because treatment arms are not always performed according to plan. We, therefore, allowed patients to choose whether to undergo NAC or surgery alone after informing the patients of the tumor staging, merits, and demerits of each treatment and the potential significance of the present trial. Finally, we did not evaluate the recurrence and disease-free survival in this study. As we know, it is also an important endpoint especially in the outcome of malignant tumor. Despite these limitations, this prospective nonrandomized study was based on a specific group of patients diagnosed with cT4 ESCC, and our treatments were protocol based, limiting potential bias.

CONCLUSIONS

NAC with irinotecan and nedaplatin in a single cycle as a new NAC regimen followed by esophagectomy on cT4 resectable ESCC is safe and effective.

ACKNOWLEDGEMENTS

This work was supported by the Fund Project of Sichuan Provincial Department of Education, Grant No. 14ZB0203 and North Sichuan Medical College Key Laboratory Open Fund. Grant No. CBY14-A-ZD09. We appreciate the contribution and the valuable assistance of Dr. Shuliang Wei and Dr. Yinglong Lai of the Cardiothoracic Surgery Department, Affiliated Hospital of North Sichuan Medical College.

References

1 Shimada H, Shiratori T, Okazumi S *et al.* Have surgical outcomes of pathologic T4 esophageal squamous cell carcinoma really improved? Analysis of 268 cases during 45 years of experience. J Am Coll Surg 2008; 206: 48–56.
2 Tachimori Y, Ozawa S, Numasaki H *et al.* Comprehensive registry of esophageal cancer in Japan, 2009. Esophagus 2016; 13: 110–37.
3 Chak A, Canto M, Gerdes H *et al.* Prognosis of esophageal cancers preoperatively staged to be locally invasive (T4) by endoscopic ultrasound (EUS): a multicenter retrospective cohort study. Gastrointest Endosc 1995; 42: 501–6.
4 Gebski V, Burmeister B, Smithers B M *et al.* Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. Lancet Oncol 2007; 8: 226–34.
5 Makino T, Doki Y. Treatment of T4 esophageal cancer. Definitive chemo-radiotherapy vs chemo-radiotherapy followed by surgery. Ann Thorac Cardiovasc Surg 2011; 17: 221–8.
6 Fujita H, Sueyoshi S, Tanaka T *et al.* Esophagectomy: is it necessary after chemoradiotherapy for a locally advanced T4 esophageal cancer? Prospective nonrandomized trial comparing

chemoradiotherapy with surgery versus without surgery. World J Surg 2005; 29: 25–31; discussion-1.
7 Brucher B L, Becker K, Lordick F *et al.* The clinical impact of histopathologic response assessment by residual tumor cell quantification in esophageal squamous cell carcinomas. Cancer 2006; 106: 2119–27.
8 Meguid R A, Hooker C M, Taylor J T *et al.* Recurrence after neoadjuvant chemoradiation and surgery for esophageal cancer: does the pattern of recurrence differ for patients with complete response and those with partial or no response? J Thorac Cardiovasc Surg 2009; 138: 1309–17.
9 Hsu F M, Lin C C, Lee J M *et al.* Improved local control by surgery and paclitaxel-based chemoradiation for esophageal squamous cell carcinoma: results of a retrospective non-randomized study. J Surg Oncol 2008; 98: 34–41.
10 Roof K S, Coen J, Lynch T J *et al.* Concurrent cisplatin, 5-FU, paclitaxel, and radiation therapy in patients with locally advanced esophageal cancer. J Radiat Oncol Biol Phys 2006; 65: 1120–8.
11 Donahue J M, Nichols F C, Li Z *et al.* Complete pathologic response after neoadjuvant chemoradiotherapy for esophageal cancer is associated with enhanced survival. Ann Thorac Surg 2009; 87: 392–9; discussion 8-9.
12 Matsubara T, Ueda M, Kokudo N, Takahashi T, Muto T, Yanagisawa A. Role of esophagectomy in treatment of esophageal carcinoma with clinical evidence of adjacent organ invasion. World J Surg 2001; 25: 279–84.
13 Tachibana M, Dhar D K, Kinugasa S *et al.* Surgical treatment for locally advanced (T4) squamous cell carcinoma of the thoracic esophagus. Dysphagia 2002; 17: 255–61.
14 Chen M F, Chen P T, Lu M S, Lee C P, Chen W C. Survival benefit of surgery to patients with esophageal squamous cell carcinoma. Sci Rep 2017; 7: 46139.
15 Akutsu Y, Kono T, Uesato M *et al.* Is the outcome of a salvage surgery for T4 thoracic esophageal squamous cell carcinoma really poor? World J Surg 2014; 38: 2891–7.
16 Shimoji H, Karimata H, Nagahama M, Nishimaki T. Induction chemotherapy or chemoradiotherapy followed by radical esophagectomy for T4 esophageal cancer: results of a prospective cohort study. World J Surg 2013; 37: 2180–8.
17 Pimiento J M, Weber J, Hoffe S E *et al.* Outcomes associated with surgery for T4 esophageal cancer. Ann Surg Oncol 2013; 20: 2706–12.
18 Seto Y, Chin K, Gomi K *et al.* Treatment of thoracic esophageal carcinoma invading adjacent structures. Cancer Sci 2007; 98: 937–42.
19 Kuroda J, Yoshida M, Kitajima M *et al.* Utility of preoperative chemoradiotherapy for advanced esophageal carcinoma. J Gastroenterol Hepatol 2012; 27: 88–94.
20 Ando N, Kato H, Igaki H *et al.* A randomized trial comparing postoperative adjuvant chemotherapy with cisplatin and 5-fluorouracil versus preoperative chemotherapy for localized advanced squamous cell carcinoma of the thoracic esophagus (JCOG9907). Ann Surg Oncol 2012; 19: 68–74.
21 Blum Murphy M A, Elimova E, Ajani J A. Current concepts and future potential in neoadjuvant chemotherapy for esophageal cancer. Expert Rev Gastroenterol Hepatol 2016; 10: 383–92.
22 Boonstra J J, Kok T C, Wijnhoven B P *et al.* Chemotherapy followed by surgery versus surgery alone in patients with resectable oesophageal squamous cell carcinoma: long-term results of a randomized controlled trial. BMC Cancer 2011; 11: 181.
23 Hara H, Tahara M, Daiko H *et al.* Phase II feasibility study of preoperative chemotherapy with docetaxel, cisplatin, and fluorouracil for esophageal squamous cell carcinoma. Cancer Sci 2013; 104: 1455–60.
24 Hirao M, Ando N, Tsujinaka T *et al.* Influence of preoperative chemotherapy for advanced thoracic oesophageal squamous cell carcinoma on perioperative complications. Br J Surg 2011; 98: 1735–41.
25 Hironaka S, Tsubosa Y, Mizusawa J *et al.* Phase I/II trial of 2-weekly docetaxel combined with cisplatin plus fluorouracil in metastatic esophageal cancer (JCOG0807). Cancer Sci 2014; 105: 1189–95.
26 Ilson D H. Phase II trial of weekly irinotecan/cisplatin in advanced esophageal cancer. Oncology (Williston Park) 2004; 18: 22–5.
27 Ilson D H, Saltz L, Enzinger P *et al.* Phase II trial of weekly irinotecan plus cisplatin in advanced esophageal cancer. J Clin Oncol 1999; 17: 3270–5.
28 Kelsen D P, Ginsberg R, Pajak T F *et al.* Chemotherapy followed by surgery compared with surgery alone for localized esophageal cancer. N Engl J Med 1998; 339: 1979–84.
29 Korst R J, Kansler A L, Port J L, Lee P C, Kerem Y, Altorki N K. Downstaging of T or N predicts long-term survival after preoperative chemotherapy and radical resection for esophageal carcinoma. Ann Thorac Surg 2006; 82: 480–5; discussion 4–5.
30 Ilson D H, Bains M, Kelsen D P *et al.* Phase I trial of escalating-dose irinotecan given weekly with cisplatin and concurrent radiotherapy in locally advanced esophageal cancer. J Clin Oncol 2003; 21: 2926–32.
31 Knox J J, Wong R, Visbal A L *et al.* Phase 2 trial of preoperative irinotecan plus cisplatin and conformal radiotherapy, followed by surgery for esophageal cancer. Cancer 2010; 116: 4023–32.
32 Janjigian Y Y, Ku G Y, Campbell J C *et al.* Phase II trial of cetuximab plus cisplatin and irinotecan in patients with cisplatin and irinotecan-refractory metastatic esophagogastric cancer. Am J Clin Oncol 2014; 37: 126–30.
33 Karagiannis G S, Pastoriza J M, Wang Y *et al.* Neoadjuvant chemotherapy induces breast cancer metastasis through a TMEM-mediated mechanism. Sci Transl Med 2017; 9: eaan0026.
34 Fujiwara Y, Lee S, Kishida S *et al.* Influence of incomplete neoadjuvant chemotherapy on esophageal carcinoma. Int J Clin Oncol 2018. doi: 10.1007/s10147-018-1291-6.
35 Rice T W, Gress D M, Patil D T, Hofstetter W L, Kelsen D P, Blackstone E H. Cancer of the esophagus and esophagogastric junction-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin 2017; 67: 304–17.
36 Zagars G K, Ballo M T, Pisters P W, Pollock R E, Patel S R, Benjamin R S. Surgical margins and reresection in the management of patients with soft tissue sarcoma using conservative surgery and radiation therapy. Cancer 2003; 97: 2544–53.
37 Chen A P, Setser A, Anadkat M J *et al.* Grading dermatologic adverse events of cancer treatments: the Common Terminology Criteria for Adverse Events Version 4.0. J Am Acad Dermatol 2012; 67: 1025–39.
38 Karimata H, Shimoji H, Nishimaki T. Clinicopathological factors predicting R0 resection and long-term survival after esophagectomy in patients with T4 esophageal cancer undergoing induction chemotherapy or chemoradiotherapy. Surg Today 2015; 45: 479–86.
39 Mariette C, Finzi L, Fabre S, Balon J M, Van Seuningen I, Triboulet J P. Factors predictive of complete resection of operable esophageal cancer: a prospective study. Ann Thorac Surg 2003; 75: 1720–6.
40 Rigas J R, Dragnev K H, Bubis J A. Docetaxel in the treatment of esophageal cancer. Semin Oncol 2005; 32: 39–51.
41 Samson P, Robinson C, Bradley J *et al.* Neoadjuvant chemotherapy versus chemoradiation prior to esophagectomy: impact on rate of complete pathologic response and survival in esophageal cancer patients. J Thorac Oncol 2016; 11: 2227–37.
42 Chi Y K, Chen Y, Li X T, Sun Y S. Prognostic significance of the size and number of lymph nodes on pre and post neoadjuvant chemotherapy CT in patients with pN0 esophageal squamous cell carcinoma: a 5-year follow-up study. Oncotarget 2017; 8: 61662–73.
43 Ilson D H, Minsky B. Irinotecan in esophageal cancer. Oncology (Williston Park) 2003; 17: 32–6.
44 Lee M S, Mamon H J, Hong T S *et al.* Preoperative cetuximab, irinotecan, cisplatin, and radiation therapy for patients with locally advanced esophageal cancer. Oncologist 2013; 18: 281–7.
45 Takigawa N, Segawa Y, Ueoka H *et al.* Combination of nedaplatin and vindesine for treatment of relapsed or refractory non-small-cell lung cancer. Cancer Chemother Pharmacol 2000; 46: 272–8.
46 Ando N, Ozawa S, Kitajima M, Iizuka T. Chemotherapy and multimodality therapy in the treatment of esophageal cancer. Gan To Kagaku Ryoho 1995; 22: 869–76.
47 Law S, Fok M, Chow S, Chu K M, Wong J. Preoperative chemotherapy versus surgical therapy alone for squamous cell

carcinoma of the esophagus: a prospective randomized trial. J Thorac Cardiovasc Surg 1997; 114: 210–7.
48 Motoori M, Yano M, Yasuda T *et al.* Early response to neoadjuvant chemotherapy in advanced esophageal cancer evaluated by computed tomography predicts the utility of a second cycle of chemotherapy. Mol Clin Oncol 2013; 1: 521–6.
49 Hamai Y, Hihara J, Emi M *et al.* Results of neoadjuvant chemoradiotherapy with docetaxel and 5-Fluorouracil followed by esophagectomy to treat locally advanced esophageal cancer. Ann Thorac Surg 2015; 99: 1887–93.
50 Naritaka Y, Ogawa K, Shimakawa T *et al.* Case report: a young woman with advanced esophageal cancer showing pathological complete response to neoadjuvant chemotherapy (CDDP, 5-FU and ADM). Anticancer Res 2004; 24: 2385–9.
51 Azria D, Ychou M, Senesse P, Dubois J B. Neoadjuvant treatment of operable esophageal cancers. Gastroenterol Clin Biol 1999; 23: 1107–8.
52 Takahashi H, Arimura Y, Yamashita K *et al.* Phase I/II study of docetaxel/cisplatin/fluorouracil combination chemotherapy against metastatic esophageal squamous cell carcinoma. J Thorac Oncol 2010; 5: 122–8.
53 Osaka Y, Shinohara M, Hoshino S *et al.* Phase II study of combined chemotherapy with docetaxel, CDDP and 5-FU for highly advanced esophageal cancer. Anticancer Res 2011; 31: 633–8.
54 Yamasaki M, Miyata H, Tanaka K *et al.* Multicenter phase I/II study of docetaxel, cisplatin and fluorouracil combination chemotherapy in patients with advanced or recurrent squamous cell carcinoma of the esophagus. Oncology 2011; 80: 307–13.
55 Tamura S, Imano M, Takiuchi H *et al.* Phase II study of docetaxel, cisplatin and 5-fluorouracil (DCF) for metastatic esophageal cancer (OGSG 0403). Anticancer Res 2012; 32: 1403–8.
56 Ferri L E, Ades S, Alcindor T *et al.* Perioperative docetaxel, cisplatin, and 5-fluorouracil (DCF) for locally advanced esophageal and gastric adenocarcinoma: a multicenter phase II trial. Ann Oncol 2012; 23: 1512–7.
57 Watanabe M, Baba Y, Yoshida N *et al.* Outcomes of preoperative chemotherapy with docetaxel, cisplatin, and 5-fluorouracil followed by esophagectomy in patients with resectable node-positive esophageal cancer. Ann Surg Oncol 2014; 21: 2838–44.
58 Kranzfelder M, Schuster T, Geinitz H, Friess H, Buchler P. Meta-analysis of neoadjuvant treatment modalities and definitive non-surgical therapy for oesophageal squamous cell cancer. Br J Surg 2011; 98: 768–83.
59 Tanaka E, Okabe H, Tsunoda S *et al.* Feasibility of thoracoscopic esophagectomy after neoadjuvant chemotherapy. Asian J Endosc Surg 2012; 5: 111–7.
60 Teniere P, Hay J M, Fingerhut A, Fagniez P L. Postoperative radiation therapy does not increase survival after curative resection for squamous cell carcinoma of the middle and lower esophagus as shown by a multicenter controlled trial. French University Association for Surgical Research. Surg Gynecol Obstet 1991; 173: 123–30.
61 Pouliquen X, Levard H, Hay J M, McGee K, Fingerhut A, Langlois-Zantin O. 5-Fluorouracil and cisplatin therapy after palliative surgical resection of squamous cell carcinoma of the esophagus. Ann Surg 1996; 223: 127–33.
62 Ando N, Iizuka T, Ide H *et al.* Surgery plus chemotherapy compared with surgery alone for localized squamous cell carcinoma of the thoracic esophagus: a Japan clinical oncology group study–JCOG9204. J Clin Oncol 2003; 21: 4592–6.
63 Chan A C, Lee D W, Griffith J F *et al.* The clinical efficacy of neoadjuvant chemotherapy in squamous esophageal cancer: a prospective nonrandomized study of pulse and continuous-infusion regimens with cisplatin and 5-Fluorouracil. Ann Surg Oncol 2002; 9: 617–24.
64 Medical Research Council Oesophageal Cancer Working G. Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. Lancet North Am Ed 2002; 359: 1727–33.
65 Pennathur A, Luketich J D, Landreneau R J *et al.* Long-term results of a phase II trial of neoadjuvant chemotherapy followed by esophagectomy for locally advanced esophageal neoplasm. Ann Thorac Surg 2008; 85: 1930–7; discussion 6-7.
66 van Hagen P, Hulshof M C, van Lanschot J J *et al.* Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012; 366: 2074–84.
67 Herskovic A, Russell W, Liptay M, Fidler M J, Al-Sarraf M. Esophageal carcinoma advances in treatment results for locally advanced disease: review. Ann Oncol 2012; 23: 1095–103.
68 Miyata H, Yamasaki M, Kurokawa Y *et al.* Clinical relevance of induction triplet chemotherapy for esophageal cancer invading adjacent organs. J Surg Oncol 2012; 106: 441–7.

EXPERIENCE FROM AN AMERICAN ASSOCIATION FOR THORACIC SURGERY FOUNDATION FOR THORACIC SURGERY TRAINING FELLOWSHIP: LUNG TRANSPLANTATION IN TORONTO GENERAL HOSPITAL

To the Editor:

Because I (D.T.) am extremely interested in lung transplantation, I applied for an American Association for Thoracic Surgery (AATS) Graham Foundation for Thoracic Surgery Training Fellowship. The goal of this fellowship is to provide an international training and educational experience for young thoracic surgeons from mainland China by having them spend a focused period of between 1 month and 1 year studying clinical techniques at a host training site in North America. Thanks to recommendation from Dr Hiroshi Date, chief of the thoracic surgery department at the Kyoto University Hospital, and acceptance by Dr Shaf Keshavjee, surgeon in chief of the University Health Network, I was fortunate to be selected by the AATS Graham Foundation to receive a Thoracic Surgery Training Fellowship, which took place with Dr Shaf Keshavjee at Toronto General Hospital (TGH) from January to March 2018.

TGH has a history of turning heparin, insulin, and pacemakers into world firsts. Beginning with the world's first successful lung transplant in 1983 and continuing with the first successful double-lung transplant in 1986,[1,2] the Lung Transplantation Program has completed more than 2000 lung transplants. In 2017 alone, about 170 lung transplants were completed at TGH, with only about 3% mortality. Dr Shaf Keshavjee has been part of many firsts of lung transplantation history at TGH. His development of a lung preservation solution that boosted the patient survival for single-lung transplants from 50% to more than 90%—is now a world standard. Another innovation of his team was the technique of ex vivo lung perfusion (EVLP), which allows lungs to be preserved at body temperature for 12 to 18 hours.[3] With the advent of EVLP, marginal donor lungs can be monitored and assessed individually to help transplant surgeons select lungs that are suitable for transplantation.[4] In February, the American Society of Transplantation awarded the Toronto Lung Transplant Program the American Society of Transplantation Innovation Award for 2018 for the clinical translation of EVLP.

I visited Dr Keshavjee in his office when I arrived at Toronto (Figure 1). He asked me many details about what I wanted to learn in TGH and gave me great encouragement and suggestions for my future research plan about lung transplantation. In addition, we talked about the current situation of lung transplantation in China. I felt that Dr Keshavjee was an example of just what my future aspirations are.

FIGURE 1. Shaf Keshavjee, MD, MSc, FRCSC, FACS (*left*), and Dong Tian, MD (*right*).

I started my fellowship when I left Dr Keshavjee's office. Studying in the Lung Transplantation Program, I experienced an unusually busy 2-month period. I shadowed Lung Transplantation Program fellows and observed about 5 lung retrievals (both donor after cardiac death and donor after brain death organs) and 15 single-lung and double lung transplants. I joined 20 meetings and lectures, looked around the medical surgical intensive care unit and general ward, observed EVLP procedures, and so on. I also visited the Latner Thoracic Surgery Research Laboratory, where I was able to observe the Lung Transplantation Program's translational research efforts involving animal lung transplantation. I gained a lot of knowledge of lung transplantation from staff surgeons and fellows during the 2- month experience. More importantly, I am more interested in lung transplantation than ever before. That enthusiasm will be important in my future time at The University of Tokyo Hospital as a PhD researcher.

In conclusion, I am very grateful to the AATS Graham Foundation for Thoracic Surgery Training Fellowship for providing me this great opportunity to visit TGH. I would like to point out that this fellowship really gave me so many wonderful treasures in TGH, and an unforgettable experience. I thank Dr Keshavjee and his lung

S.K. is Chief Scientific Officer of Perfusix Canada and XOR Labs Toronto. D.T. has nothing to disclose with regard to commercial support.

transplantation team for this valuable learning opportunity and for giving me a lot of support during this fellowship.

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The Thoracic Surgery Training Fellowship was funded by American Association for Thoracic Surgery.

References

1. Cooper JD, Pearson FG, Patterson GA, Todd TR, Ginsberg RJ, Goldberg M, et al. Technique of successful lung transplantation in humans. *J Thorac Cardiovasc Surg*. 1987;93:173-81.
2. Patterson GA, Cooper JD, Goldman B, Weisel RD, Pearson FG, Waters PF, et al. Technique of successful clinical double-lung transplantation. *Ann Thorac Surg*. 1988;45:626-33.
3. Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, et al. Technique for prolonged normothermic ex vivo lung perfusion. *J Heart Lung Transplant*. 2008;27:1319-25.
4. Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med*. 2011;364:1431-40.

https://doi.org/10.1016/j.jtcvs.2018.04.036

日中笹川医学奨学金制度(学位取得コース)中間報告書
研究者用

Supported by 日本財団 THE NIPPON FOUNDATION

第40期　　研究者番号：G4005　　作成日：2019年 3月 4 日

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研究テーマ	Length from pylorus ring to distal edge of the tumor in combinations with tumor location are powerful determinants of nodal metastasis along the distal portion of the stomach 腫瘍の位置と組み合わせた幽門輪から腫瘍の遠位端までの長さは胃の遠位部に沿ったリンパ節転移の強力な決定因子である				
専攻種別	論文博士	□	課程博士	☑	

1. 研究概要

1) 目的 (Goal)

Proximal gastrectomy has been indicated by tumor location (upper third of stomach) and T stage (T1 stage) for nodal negative (N0) patients according to the Japanese gastric cancer treatment guidelines. However, in clinical practice, some patients with proximal cancers underwent total gastrectomy were proved with no nodal metastasis along the distal portion of the stomach (No.3b, 4d, 5, and 6), even for cT2 and cN+ cases, postoperatively. Furthermore, stomach sizes of every patients varied from each other. Therefore, the length from pylorus ring to distal edge of the tumor (LPD) also varied among patients with proximal tumors. Tumors locating in upper third stomach with longer LPD may have lower risk of nodal metastasis along the distal portion. Therefore, it remains unclear whether the indication of proximal gastrectomy could be extended or not. This study was conducted to invest a powerful risk factor to predict nodal metastasis along the distal portion of the stomach, and to identify if we could expend the indications of proximal gastrectomy.

2) 戦略 (Approach)

To invest the risk factors of peri-gastric nodal metastasis, we only included patients with total gastrectomy. We try to find powerful risk factors to predict nodal metastasis along the distal portion of the stomach, and to identify if we could expend indications of proximal gastrectomy.

3) 患者と方法 (Patients and Methods)

Between Jan 2000 to Dec 2013, 445 patients were identified as gastric cancer and underwent total gastrectomy in the Department of Gastrointestinal Surgery of Tokyo University Hospital. The univariate and multivariable analysis of prognostic factors for the whole population was conducted. The mean number of peri-gastric lymph nodes examined and metastasis rates were compared between upper, middle and lower third tumors. Multivariable logistic regression analysis was conducted to confirm the predictors of nodal metastasis of no.3b, 4d, 5, and 6 lymph nodes.

4) 結果 (Results)

The univariate analysis identified age ($p = 0.038$), tumor size ($p < 0.001$), tumor location ($p = 0.001$), AJCC 8th pT stage ($p < 0.001$), AJCC 8th pN stage ($p < 0.001$), adequate or inadequate lymph node examined ($p < 0.001$), lymphatic invasion ($p < 0.001$), venous invasion ($p < 0.001$), adjuvant chemotherapy ($p < 0.001$) to significantly correlate with prognosis. Multivariate analysis demonstrated that age (OR, 1.03, 95% CI, 1.01-1.04, $p < 0.001$), AJCC 8th pT stage (OR, 1.57, 95% CI, 1.30-1.90, $p < 0.001$), AJCC 8th pN stage (OR, 1.39, 95% CI, 1.19-1.63, $p < 0.001$), and adequate or inadequate lymph node examined (OR, 8.24, 95% CI, 4.71-14.42, $p < 0.001$) were independent prognostic factors. Tumor size (OR, 0.76, 95% CI, 0.60-0.98, $P = 0.031$) and LPD (OR, 0.82, 95% CI, 0.68-0.99, $P = 0.038$) were identified as risk predictors of no.3b nodal metastasis. Gender (OR, 2.38, 95% CI, 1.29-4.39, $P = 0.005$), tumor location ($P = 0.001$), and AJCC 8th T stage ($P < 0.001$) were proved risk predictors of no.4d nodal metastasis. LPD (OR, 0.82, 95% CI, 0.70-0.95, $P = 0.007$) was confirmed as the only risk predictor of no.5 nodal metastasis. Tumor location ($P = 0.002$), LPD (OR, 0.78, 95% CI, 0.69-0.89, $P < 0.001$), and AJCC 8th T stage ($P = 0.02$) were identified as risk predictors of no.6 nodal metastasis.

5) 考察 (Discussion)

According to our data, the metastasis rates of upper third tumors were 11.1%, 2.3% and 2.3% for No.4d, 5 and 6 lymph node stations, respectively. The metastasis rates of no. 4d, 5 and 6 lymph node stations were 0.0% for T1-2 cases of upper third tumors, however, the total number was small. Multivariable logistic regression analysis suggested that LPD was a powerful determinant of no.3b, no.5 and no.6 nodal metastasis of gastric cancer. LPD may be a powerful determinant of nodal metastasis along the distal portion of stomach. LPD in combination with tumor location should be indicated as powerful determinants for proximal gastrectomy, and additional predicters including tumor size, AJCC 8th T stage, and gender should also be taken into considerations. Further studies should be conducted to identify the optimal cutoff value.

6) 参考文献 (References)

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019; 9(1): 7-34.
[2] Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer. 2017; 20(1): 1-19.
[3] Haruta S, Shinohara H, Hosogi H, Ohkura Y, Kobayashi N, Mizuno A, Okamura R, Ueno M, Sakai Y, Udagawa H. Proximal gastrectomy with exclusion of no. 3b lesser curvature lymph node dissection could be indicated for patients with advanced upper-third

Table 3. Comparison of number of peri-gastric lymph node examined between upper, middle and lower third tumors. (N = 445)

Station	Upper third (n = 262)		Middle third (n = 138)		Lower third (n = 45)	
	Mean	SD	Mean	SD	Mean	SD
No.1	5.17	4.17	4.60	4.48	4.40	4.025
No.2	2.94	2.91	2.33	2.54	2.29	2.96
No.3	6.67	6.20	5.64	6.31	5.58	5.65
No.3a*	6.66	5.19	6.65	4.39	5.50	2.72
No.3b*	1.47	2.19	3.03	3.82	0.80	1.23
No.4sa	1.85	2.43	2.28	4.18	1.49	1.90
No.4sb	2.71	3.44	2.50	3.31	2.16	2.51
No.4d	7.15	6.24	7.50	6.07	5.49	4.69
No.5	0.68	1.02	0.64	1.25	0.64	0.93
No.6	4.15	3.09	4.43	3.80	4.89	3.17

Table 4 Comparison of peri-gastric lymph node metastasis rates between upper, middle, and lower third tumors. (N = 445)

Station	Upper third (n = 262)		Middle third (n = 138)		Lower third (n = 45)	
	Positive/negative cases	Metastasis rate	Positive/negative cases	Metastasis rate	Positive/negative cases	Metastasis rate
No.1	76/186	29.0%	43/95	31.2%	14/31	31.1%
No.2	48/214	18.3%	14/124	10.1%	4/41	8.9%
No.3	85/177	32.4%	46/92	33.3%	16/29	33.3%
No.3a*	26/59	9.9%	14/17	45.2%	5/5	50.0%
No.3b*	8/77	9.4%	4/27	12.9%	2/8	20.0%
No.4sa	19/243	7.3%	15/123	10.9%	2/43	4.4%
No.4sb	24/238	9.2%	21/117	15.2%	7/38	15.6%
No.4d	29/233	11.1%	47/91	34.1%	16/29	35.6%
No.5	6/256	2.3%	14/124	10.1%	5/40	11.1%
No.6	6/256	2.3%	29/109	21.0%	16/29	33.3%

Table 5. Number of node-positive cases according to depth of invasion (T stage) and peri-gastric lymph node stations. (N = 445)

Station	Upper third, n/total					Middle third, n/total					Lower third, n/total				
	T1	T2	T3	T4	Total	T1	T2	T3	T4	Total	T1	T2	T3	T4	Total
No.1	5/74	3/26	27/74	41/88	88/262	2/38	2/7	6/27	33/66	43/138	1/8	0/2	2/9	11/26	14/45
No.2	0/74	3/26	14/74	31/88	48/262	0/38	0/7	1/27	13/66	14/138	0/8	0/2	1/9	3/26	4/45
No.3	6/74	11/26	30/74	38/88	85/262	3/38	1/7	8/27	34/66	46/138	0/8	0/2	2/9	14/26	16/45
No.3a*	8/25	0/5	4/28	14/27	26/85	4/8	1/3	3/6	6/14	14/31	1/2	—	0/2	4/6	5/10
No.3b*	4/25	0/5	2/28	2/27	8/85	0/8	0/3	0/6	4/14	4/31	0/2	—	0/2	2/6	2/10
No.4sa	0/74	0/26	2/74	17/88	19/262	1/38	0/7	3/27	11/66	15/138	0/8	0/2	0/9	2/26	2/45
No.4sb	0/74	0/26	4/74	20/88	24/262	0/38	0/7	5/27	16/66	21/138	0/8	0/2	1/9	6/26	7/45
No.4d	0/74	0/26	6/74	23/88	29/262	3/38	1/7	8/27	35/66	47/138	0/8	0/2	2/9	14/26	16/45
No.5	0/74	0/26	1/74	5/88	6/262	1/38	0/7	1/27	12/66	14/138	0/8	0/2	1/9	4/26	5/45
No.6	0/74	0/26	3/74	3/88	6/262	1/38	0/7	3/27	25/66	29/138	0/8	1/2	3/9	12/26	16/45

Table 6. Multivariable logistic regression analysis of predictors of no. 3b, 4d, 5, and 6 nodal metastases.

Factors	No.3d (n = 126)		No.4d (n = 445)		No.5 (n = 445)		No.6 (n = 445)	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Gender (male)	0.65 (0.13–3.42)	0.617	2.38 (1.29–4.39)	**0.005**	0.95 (0.35–2.56)	0.915	1.42 (0.63–3.20)	0.400
Age, years	1.05 (0.97–1.13)	0.234	1.01 (0.98–1.03)	0.575	1.00 (0.96–1.04)	0.870	1.01 (0.98–1.04)	0.569
Tumor size, cm	0.76 (0.60–0.98)	**0.031**	1.04 (0.97–1.12)	0.230	0.94 (0.84–1.06)	0.315	0.96 (0.87–1.05)	0.343
Tumor location		0.914		**0.001**		0.220		**0.002**
Upper third	1.00		1.00		1.00		1.00	
Middle third	0.93 (0.18–4.73)	0.932	3.43 (1.77–6.63)	<0.001	2.22 (0.76–6.53)	0.147	5.88 (2.20–15.73)	<0.001
Lower third	1.52 (0.14–16.11)	0.727	2.71 (0.99–7.38)	0.052	1.06 (0.24–4.71)	0.943	5.54 (1.61–19.03)	0.007
LDMP, cm	0.82 (0.68–0.99)	**0.038**	0.95 (0.88–1.02)	0.147	0.82 (0.70–0.95)	**0.007**	0.78 (0.69–0.89)	**<0.001**
Lauren classification		0.053		0.303		0.419		0.542
Intestinal type	1.00		1.00		1.00		1.00	
Diffuse type	2738695335	1.00	2.59 (0.87–7.74)	0.089	1.08 (0.17–6.97)	0.935	1.88 (0.46–7.77)	0.383
Mixed type	223711590.3	1.00	2.62 (0.96–7.12)	0.059	2.39 (0.47–12.18)	0.295	2.47 (0.69–8.80)	0.163
Unknown	6.65	1.00	0.00	0.999	0.00	0.999	0.00	0.999
AJCC 8th T stage		0.779		**<0.001**		0.054		**0.020**
T1	1.00		1.00		1.00		1.00	
T2	0.00	1.00	1.34 (0.13–13.78)	0.808	0.00	0.998	5.30 (0.27–103.49)	0.271
T3	0.40 (0.05–3.18)	0.38	6.52 (1.76–24.11)	0.005	3.39 (0.33–35.21)	0.307	11.46 (1.33–99.08)	0.027
T4	1.01 (0.15–6.72)	0.99	15.08 (4.29–53.07)	<0.001	11.86 (1.41–100.01)	0.023	21.48 (2.63–175.19)	0.004

Table 7. Assessment of risks of patients of No.3b and No.4d nodal metastases

No.3b		No.4d	
Factors	Sensitivity	Factors	Nodal metastasis risk
LDMP, cm		Gender	Male (Low)
15.1–15.95	85.7%		Female (High)
16.35–18.75	92.9%	Tumor location	Upper (Low)
≥ 19.75	100.0%		Middle (High)
Tumor size, cm			Lower (High)
3.95–4.10	85.7%	AJCC 8th T stage	T1, T2 (Low)
2.35–3.7	92.9%		T3 (High)
≤ 2.2	100.0%		T4 (High)

Table 8. Assessment of risks of patients of No.5 and No.6 nodal metastases.

No.5		No.6	
Factor	Sensitivity	Factor	Sensitivity
LDMP, cm		LDMP, cm	
8.05–10.95	88.0%	7.15–8.25	86.3%
11.05–12.45	96.0%	8.55–9.45	92.2%
≥ 12.7	100.0%	≥ 11.05	100.0%

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title	Lymphovascular invasion as a predictor for lymph node metastasis and a prognostic factor in gastric cancer patients under 70 years of age: A retrospective analysis.																
掲載誌名 Published journal	Int J Surg (IF=2.693)																
	2018	年	5	月	56	巻（号）	31	頁	～	43	頁	言語 Language	English				
第1著者名 First author	Zhang Chun-Dong				第2著者名 Second author	Ning Fei-Long				第3著者名 Third author	Zeng Xian-tao						
その他著者名	Dai Dong-Qiu																
論文名 2 Title	Reevaluation of laparoscopic versus open distal gastrectomy for early gastric cancer in Asia: A meta-analysis of randomized controlled trials.																
掲載誌名 Published journal	Int J Surg (IF=2.693) *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*																
	2018	年	5	月	56	巻（号）	31	頁	～	43	頁	言語 Language	English				
第1著者名 First author	Zhang Chun-Dong				第2著者名 Second author	Hiroharu Yamashita				第3著者名 Third author	Zhang Shun						
その他著者名	Yasuyuki Seto																
論文名 3 Title	Comparison of different lymph node staging systems in patients with resectable colorectal cancer																
掲載誌名 Published journal	Front Oncol (IF=4.414) (** Co-first author*) *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*																
	2019	年	1	月	8	巻（号）	671	頁	～		頁	言語 Language	English				
第1著者名 First author	Pei Jun-Peng *				第2著者名 Second author	Zhang Chun-Dong *				第3著者名 Third author	Yu-Chen Fan						
その他著者名	Dai Dong-Qiu																
論文名 4 Title	Gastric Cancer Surgery: Historical Background and Perspective in Western Countries versus Japan																
掲載誌名 Published journal	Finished and preparation for submit. *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*																
		年		月		巻（号）		頁	～		頁	言語 Language	English				
第1著者名 First author	Zhang Chun-Dong				第2著者名 Second author	Hiroharu Yamashita				第3著者名 Third author	Susumu Aikou						
その他著者名	Yasuyuki Seto																
論文名 5 Title	Length from pylorus ring to distal edge of the tumor in combinations with tumor location are powerful determinants of nodal metastasis along the distal portion of the stomach																
掲載誌名 Published journal	Ongoing (Data collect finished and data analysis finished) *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*																
		年		月		巻（号）		頁	～		頁	言語 Language					
第1著者名 First author	Zhang Chun-Dong				第2著者名 Second author	Hiroharu Yamashita				第3著者名 Third author	Susumu Aikou						
その他著者名	Yasuyuki Seto																

3. 学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載してください。

※Describe your presentation as the principal presenter in major academic meetings including general meetings or international meetings.

学会名 Conference	None.							
演 題 Topic								
開催日 date	年	月	日	開催地 venue				
形式 method	□ 口頭発表 Oral	□ ポスター発表 Poster		言語 Language	□ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter								
学会名 Conference								
演 題 Topic								
開催日 date	年	月	日	開催地 venue				
形式 method	□ 口頭発表 Oral	□ ポスター発表 Poster		言語 Language	□ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter								
学会名 Conference								
演 題 Topic								
開催日 date	年	月	日	開催地 venue				
形式 method	□ 口頭発表 Oral	□ ポスター発表 Poster		言語 Language	□ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter								
学会名 Conference								
演 題 Topic								
開催日 date	年	月	日	開催地 venue				
形式 method	□ 口頭発表 Oral	□ ポスター発表 Poster		言語 Language	□ 日本語	□ 英語	□ 中国語	
共同演者名 Co-presenter								

4. 受賞（研究業績） Award (Research achievement)

名 称 Award name	None.			
	国名 Country		受賞年 Year of	年 月
名 称 Award name				
	国名 Country		受賞年 Year of	年 月

5．本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	□ 有 ■ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円
受給実績 Receipt record	□ 有 ■ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

6．他の奨学金受給 Another awarded scholarship

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助成機関名称 Funding agency	
奨学金名称 Scholarship name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

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発表機関 Released medium			
発表形式 Release method	・新聞 ・雑誌 ・Web site ・記者発表 ・その他（ ）		
発表タイトル Released title			

8．本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有 ■ 無	出願国 Application	
出願内容（概要） Application contents			

9．その他 Others

(1)2018/04—2019/02 University of Tokyo Hospital, Department of Gastrointestinal Surgery: Advanced Clinical Training and clinical research.
(2) 2019/02—Now National Cancer Center, Department of Epigenetics: Basic research: Establishment of a DNA methylation marker to identify stomach and esophagus adenocarcinoma involving the EGJ.
(3) 2019/01—2019.12 Became an editorial board member of 《Chinese Journal of General Surgery》.

指導責任者（署名）

日中笹川医学奨学金制度(学位取得コース)中間評価書
論文博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　研究者番号：G4005　作成日　：2019 年 3 月20日

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研究先（指導教官）	東京大学大学院医学系研究科　消化管外科学・乳腺内分泌外科学(瀬戸泰之　教授　)				
研究テーマ	Length from pylorus ring to distal edge of the tumor in combinations with tumor location are powerful determinants of nodal metastasis along the distal portion of the stomach. 腫瘍の位置と組み合わせた幽門輪から腫瘍の遠位端までの長さは胃の遠位部に沿ったリンパ節転移の強力な決定因子である				
専攻種別	☑論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	(優) 良　可　不可	取得単位数 取得単位数／取得すべき単位総数
学生本人が行った研究の概要	東大病院胃食道外科における 2000 年から 2013 年までに施行された胃全摘症例を用いた臨床研究である。目的は、胃上部癌に対する噴門側切除の適切な適応基準を検討することである。胃上部癌の胃下部リンパ節(No. 4d, 5, 6)への転移頻度はそれぞれ 11. 1%, 2. 3%, 2. 3%で決して高くないことが明らかにされた。しかしながら、現状一般的には胃を残せる噴門側切除ではなく、胃全摘が行われている。それは、上記リンパ節郭清が必要と考えられているからである。今回の検討では、上記リンパ節転移のリスク因子として、幽門輪から腫瘍の遠位端までの長さが挙げられた。よって、噴門側切除適応を検討する際には、幽門輪から腫瘍の遠位端までの長さを考慮にいれることが推奨されると結論づけられた。	
総合評価	【良かった点】 噴門側切除適応を検討するに際し、これまでは食道胃接合部から腫瘍遠位端までの長さが重要であるとされていたが、今回の検討では、さらに幽門輪からの距離も重要であることが明らかにされた。このような観点からの報告はこれまでなく、おそらく世界初の検討と考えられる。 【改善すべき点】 今回の検討は、あくまでも切除標本での検討であるので、臨床においては、術前に内視鏡等にて、その長さを測定する必要がある。日常診療での活用法も検討すべきと考える。 【今後の展望】 論文化することで、世に周知でき、今後のガイドライン作成においても、大いに参考になるものと考える。極めて有用な研究結果である。	
学位取得見込	今後の胃癌エピゲノム環境の検討とあわせ、学位取得は問題ないものと考える。	

評価者（指導教官名）瀬戸　泰之 (印)

International Journal of Surgery 53 (2018) 214–220

Contents lists available at ScienceDirect

International Journal of Surgery

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Lymphovascular invasion as a predictor for lymph node metastasis and a prognostic factor in gastric cancer patients under 70 years of age: A retrospective analysis

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ARTICLE INFO

Keywords:
Lymphovascular invasion
Lymph node metastasis
Survival rate
Independent prognostic factor
Resected gastric cancer

ABSTRACT

Background: Accumulating evidence has confirmed the potential prognostic value of LVI in patients with cancers. This aim of the current study was to clarify the potential relationship between LVI and lymph node metastasis, establish predictive clinicopathologic prognostic factors for LVI and lymph node metastasis, and determine the prognostic significance of LVI for patients younger than 70 years with resected gastric cancer.

Methods: Overall survival rates were calculated using Kaplan-Meier analysis. Differences in proportions of patients were tested with the χ^2 test. Univariate and multivariate analyses were applied to identify independent prognostic factors. Logistic regression analysis was employed to identify the risk factors predicting the presence of LVI and LN metastasis.

Results: Univariate analysis led to the identification of tumor size, LVI and pN stage as factors significantly correlated with prognosis. Multivariate analysis demonstrated that tumor size, LVI, pN stage, and number of LNs retrieved are independent prognostic factors for the entire population. Logistic regression analysis proved that LVI and pT stage were significantly associated with LN metastasis.

Conclusion: LVI is an independent prognostic factor predicting LN metastasis and a strongly independent predictor of survival for patients with resected gastric cancer. We recommend that LVI should be taken into account as an important adjuvant prognostic factor, specially for pN0 cases with inadequate LNs retrieved. And the maximum number of LNs possible should be retrieved for optimal staging, especially for patients with higher cT stage.

1. Introduction

Gastric cancer has emerged as a major global public health problem [1–4] with the highest incidence in China [5]. Lymphovascular invasion (LVI) is defined as tumor cell spread through the lymphatic vessels [6]. Accumulating evidence has confirmed the potential prognostic value of LVI in patients with cancer of the esophagus [7–9], adenocarcinoma of the esophagogastric junction [6], colon cancer [10], and gastric cancer [11–14].

Importantly, the majority of previous studies have included patients older than 80 or even 85 years [6–14]. However, the average lifespans of men and women in China are 74 and 77 years, respectively. Therefore, the long-term effect of curative gastrectomy for gastric cancer may not be evaluable in such elderly patients [15], and inclusion of patients within this age group may lead to unreliable results.

This aim of the current study was to clarify the potential relationship between LVI and lymph node metastasis, establish predictive clinicopathologic prognostic factors for LVI and lymph node metastasis, and determine the prognostic significance of LVI for patients younger than 70 years with resected gastric cancer.

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https://doi.org/10.1016/j.ijsu.2018.03.073
Received 21 November 2017; Received in revised form 24 February 2018; Accepted 25 March 2018
Available online 31 March 2018
1743-9191/

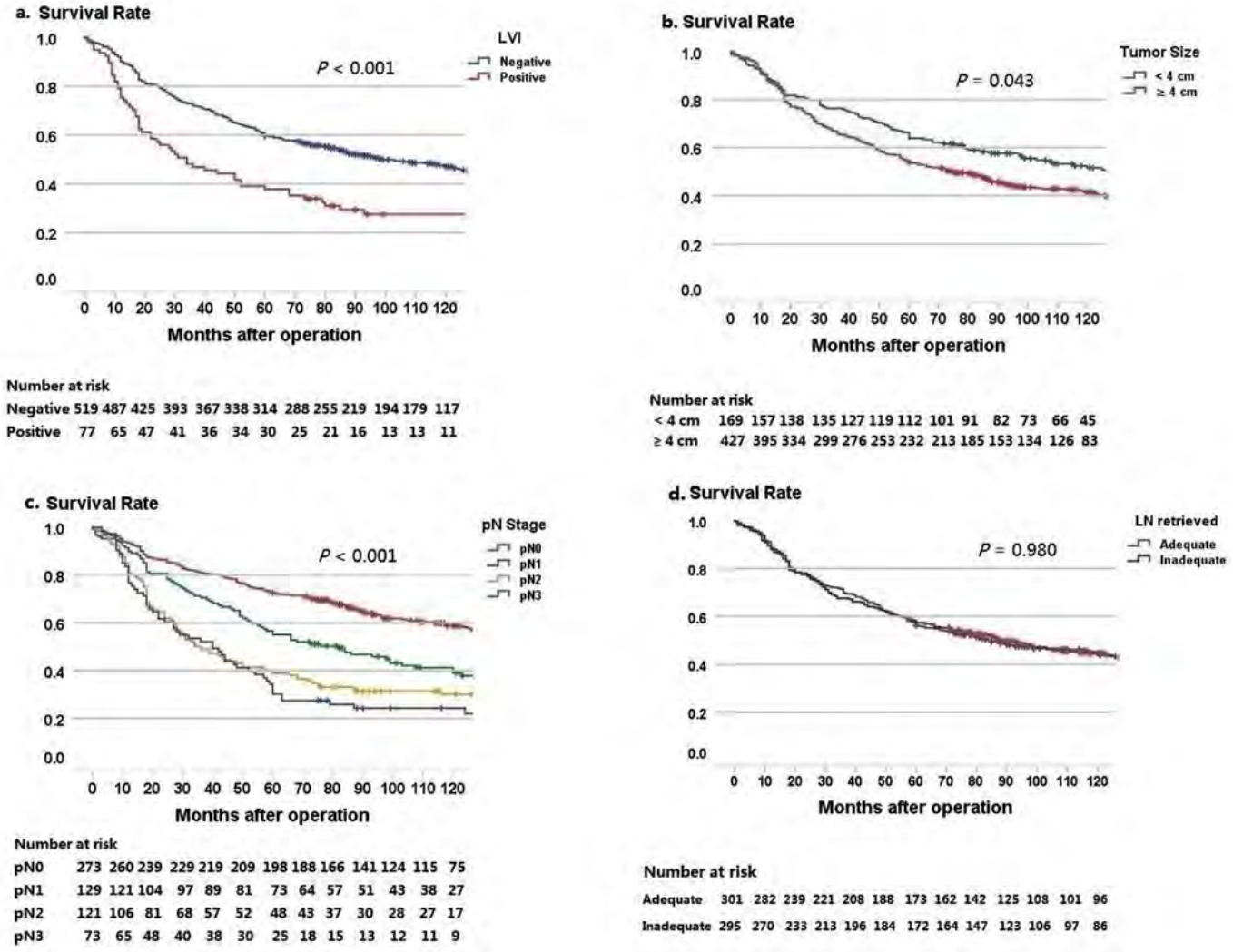

Fig. 1. Kaplan-Meier curves of the entire population according to LVI (Fig 1a), tumor size (Fig 1b), pN stage (Fig 1c), and LNs retrieved (Fig 1d).

2. Methods

Between February 1984 and February 2010, 596 patients with gastric cancer subjected to primary surgical resection in our institution were enrolled into a retrospective database. This study was approved by the Ethics Committee. All patient records and information were anonymized and de-identified prior to analysis. The work has been reported in line with the STROCSS criteria [16].

2.1. Surgical approach

All patients underwent potentially curative resection for histologically proven adenocarcinoma. Patients were subjected to total, proximal subtotal or distal subtotal gastrectomy with standard D2 (D2) or extended D2 (D2+) lymphadenectomy. Following gastrectomy, Billroth I, Billroth II or Roux-Y reconstruction was performed.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: histologically proven stomach adenocarcinoma, curative operation, negative resection margins (R0), complete medical records, D2 or D2+ lymphadenectomy, non-emergent surgery. Exclusion criteria included preoperative adjuvant therapy, laparoscopic-assisted surgery, stage IV cancer, previous or concomitant cancer, and patients over 70 years of age.

The clinicopathologic features investigated for prognostic significance included gender, age, type of anesthesia, blood loss, tumor size, reconstruction type, gastrectomy, histologic grade, depth of invasion (pT stage), number of regional LN metastases (pN stage), LVI, number of lymph nodes (LN) retrieved, recurrence or metastasis, and chemotherapy.

2.3. Pathological assessment

All specimens were analyzed by two independent and experienced pathologists, and different opinions were resolved by discussion to establish the final diagnosis. Carcinoma lesions together with the surrounding gastric wall were fixed in formalin and cut into multiple 5 mm slices in parallel with the lesser curvature. Venous invasion refers to tumor cell lining the venous endothelial surface, and tumor cell thrombi inside the lumen of the vein, which was identified by immunohistochemical staining. The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach was applied to re-stage all patients in this study. According to the current guidelines for gastric cancer, examination of at least 15 LNs is strongly recommended for adequate staging [17,18]. The pathology report mainly included data on tumor size, pT stage, pN stage, LVI, number of LNs retrieved, and histologic grade.

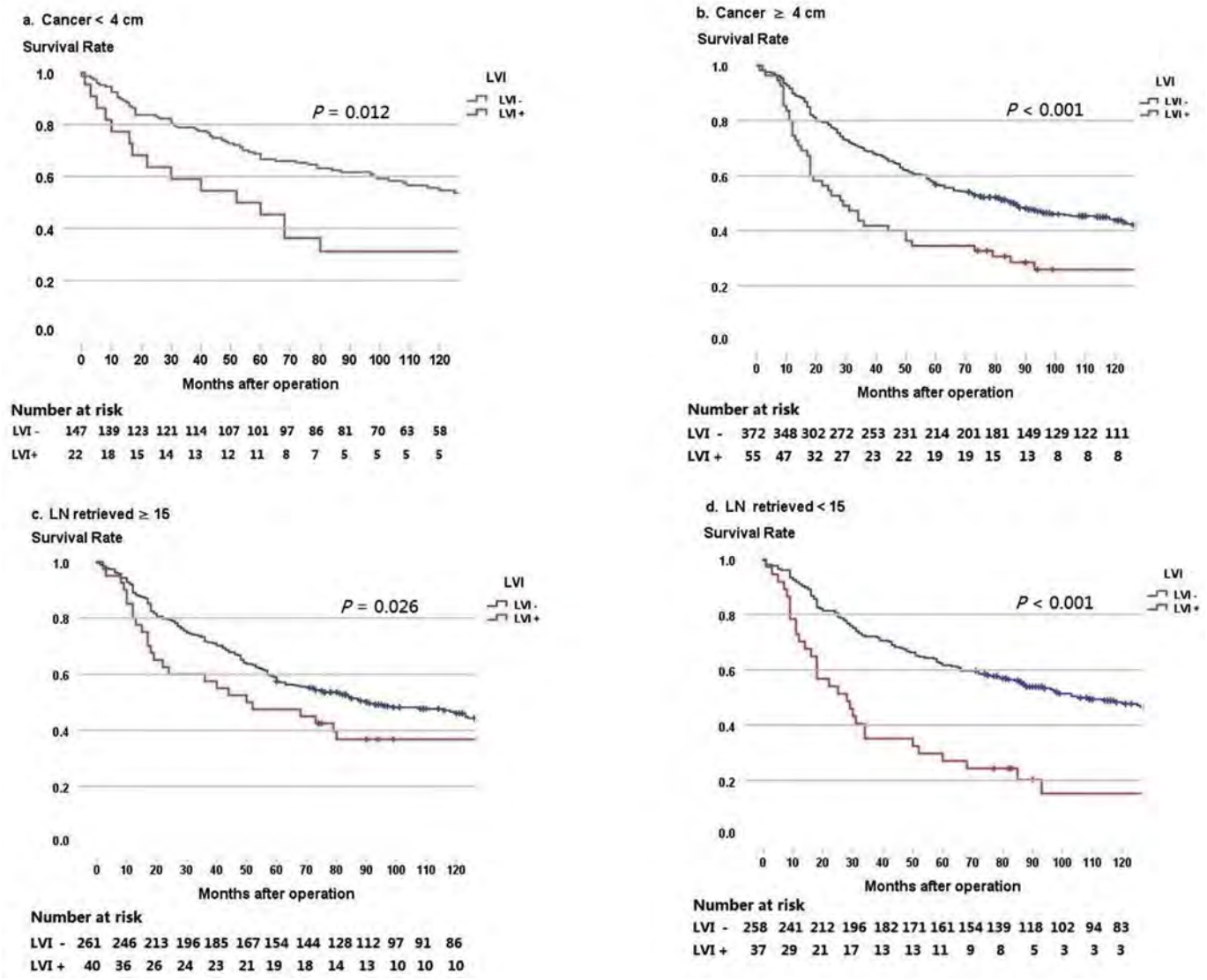

Fig. 2. Kaplan-Meier curves comparing LVI- and LVI+ patient groups with tumor sizes < 4 cm (Fig 2a), tumor sizes ≥ 4 cm (Fig 2b), LNs retrieved ≥ 15 (Fig 2c), and LNs retrieved < 15 (Fig 2d).

2.4. Follow-up

All patients were systematically followed up by personal contact with a phone call until death or the cut-off date (the final follow-up was in October 2014) over a duration of 1–368 months. The follow-up rate was 98.0%, and 12 patients were lost to follow-up in total and were excluded from this study. Complete histories were available for all patients, and physical and chemical profiles were examined every 3 months for 1–2 years, every 6–12 months for 3–5 years and annually thereafter. Overall, 596 patients younger than 70 years with resected gastric cancer were included.

2.5. Statistical analysis

Overall survival rates were calculated using Kaplan-Meier analysis including 95% confidence interval (95% CI), and examined with the log-rank test. The number at risk is shown on all Kaplan-Meier curves (Figs. 1–2). Differences in proportions of patients were tested with the χ^2 test. Univariate analysis with log-rank test and multivariate analysis were applied to identify independent prognostic factors. Logistic regression analysis was employed to identify the risk factors predicting the presence of LVI and LN metastasis. A p value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS Statistical Software (version 22.0) (SPSS, Inc., Chicago, IL, USA).

3. Results

A total of 62 patients older than 70 years (age range: 71–90 years) of age were excluded. Overall, 596 patients with resected gastric cancer were assessed for eligibility. The age range of the entire patient population was between 30 and 70 years. Among the patients examined, absence of LVI (LVI-) was confirmed in 519 and presence of LVI (LVI+) in 77 patients. Within the LVI- group, 134 (25.8%) were female and 385 (74.2%) were male. The LVI+ group comprised 22 (28.6%) female and 55 (71.4%) male patients.

The two groups (LVI- and LVI+) were balanced with respect to gender (p = 0.608), age (p = 0.931), type of anesthesia (p = 0.414), blood loss (p = 0.102), tumor size (p = 0.964), reconstruction type (p = 0.273), gastrectomy (p = 0.511), histologic grade (p = 0.413), pT stage (p = 0.740), number of LNs retrieved (p = 0.786), recurrence or metastasis (p = 0.987), and chemotherapy (p = 0.664). We observed a significant difference only in pN stage (p < 0.001) between LVI- and LVI+ groups in Table 1.

Univariate analysis led to the identification of tumor size

Table 1
Differences in clinicopathologic features in groups of patients with absence and presence of LVI subjected to gastrectomy.

Variables	LVI – *n* (%)	LVI+ *n* (%)	*p* value
Gender			0.608
Female	134 (25.8)	22 (28.6)	
Male	385 (74.2)	55 (71.4)	
Age, years			0.931
< 65	375 (72.3)	56 (72.7)	
≥ 65	144 (27.7)	21 (27.3)	
Type of anesthesia			0.414
General anesthesia	437 (84.2)	62 (80.5)	
Epidural anesthesia	82 (15.8)	15 (19.5)	
Blood loss, ml			
< 500	479 (92.3)	75 (97.4)	0.102
≥ 500	40 (7.7)	2 (2.6)	
Tumor size, cm			0.964
< 4	147 (28.3)	22 (28.6)	
≥ 4	372 (71.7)	55 (71.4)	
Reconstruction type			0.273
Billroth I	428 (82.5)	58 (75.3)	
Billroth II	72 (13.9)	16 (20.8)	
Roux-Y	19 (3.6)	3 (3.9)	
Gastrectomy			0.511
Total	39 (7.5)	5 (6.5)	
Proximal subtotal	54 (10.4)	5 (6.5)	
Distal subtotal	426 (82.1)	67 (87.0)	
Histologic grade			0.413
G1	41 (7.9)	4 (5.2)	
G2	168 (32.4)	20 (26.0)	
G3	278 (53.5)	49 (63.6)	
G4	32 (6.2)	4 (5.2)	
pT stage [a]			0.740
pT1	79 (15.2)	11 (14.3)	
pT2	126 (24.3)	17 (22.1)	
pT3	177 (34.1)	24 (31.2)	
pT4a	137 (26.4)	25 (32.4)	
pN stage [a]			< 0.001
pN0	257 (49.5)	16 (20.8)	
pN1	114 (22.0)	15 (19.5)	
pN2	96 (18.5)	25 (32.4)	
pN3	52 (10.0)	21 (27.3)	
Number of LNs retrieved			0.786
Adequate, n ≥ 15	261 (50.3)	40 (51.9)	
Inadequate, n < 15	258 (49.7)	37 (48.1)	
Recurrence or metastasis			0.987
Absent	350 (67.4)	52 (67.5)	
Present	169 (32.6)	25 (32.5)	
Chemotherapy			0.664
No	449 (86.5)	68 (88.3)	
Yes	70 (13.5)	9 (11.7)	

Two tailed *t*-tests of mean ± standard deviation (SD); *n*, number of patients; LNs, lymph nodes; LVI-, absence of lymphovascular invasion; LVI+, presence of lymphovascular invasion; G1, well differentiated; G2, moderately differentiated, G3, poorly differentiated, G4, undifferentiated.

[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

(p = 0.043), LVI (p < 0.001) and pN stage (p < 0.001) as factors significantly correlated with prognosis. All clinicopathologic factors were included in the first step of multivariate analysis, which demonstrated that tumor size (RR, 1.332; 95% CI, 1.062–1.671, p = 0.013), LVI (RR, 1.487; 95% CI, 1.122–1.971, p = 0.006), pN stage (RR, 1.413; 95% CI, 1.282–1.558, p < 0.001), and number of LNs retrieved (RR, 1.304; 95% CI, 1.057–1.608, p = 0.013) are independent prognostic factors for the entire population. In the second step of multivariate analysis, histological grade, pT stage, and chemotherapy were added. Notably, tumor size (RR, 1.319; 95% CI, 1.054–1.650, p = 0.015), LVI (RR, 1.489; 95% CI, 1.127–1.968, p = 0.005), pN stage (RR, 1.422; 95% CI, 1.290–1.566, p < 0.001), and number of LNs retrieved (RR, 1.298; 95% CI, 1.054–1.599, p = 0.014) remained independent prognostic factors. The 5-year overall survival rates (5-YSR) are presented in Table 2. Survival curves comparing LVI, tumor size, pN stage, and number of LNs retrieved are shown in Fig. 1.

Prognosis for patients in the LVI- and LVI+ groups stratified by tumor size, pN stage, LVI and number of LNs retrieved was compared. Patients in the LVI+ group had significant poorer 5-YSR than those in the LVI- group. Significant differences in 5-YSR were observed in the entire population (37.7% for LVI+ vs. 59.9% for LVI-, p < 0.001; log-rank test) as well as patients with tumor sizes less than 4 cm (45.5% for LVI+ vs. 66.7% for LVI-, p = 0.012; log-rank test), tumor sizes ≥4 cm (34.5% for LVI+ vs. 56.7% for LVI-, p < 0.001; log-rank test), adequate LNs retrieved (47.5% for LVI+ vs. 57.5% for LVI-, p = 0.026; log-rank test), and inadequate LNs retrieved (27.0% for LVI+ vs. 61.6% for LVI-, p < 0.001; log-rank test). Importantly, patients in the LVI+ group with inadequate LNs retrieved had poorer 5-YSR (27.0%) than those in the LV1- group with lymph node metastasis (73.2% for pN1 and LVI-, 57.9% for pN2 and LVI-, 40.6% for pN3 and LVI-, 30.8% for pN3 and LVI-) in Table 3. Survival curves comparing LVI+ and LVI- groups stratified by tumor size and LNs retrieved are shown in Fig. 2.

Logistic regression analysis was applied to determine the risk factors predictive of LVI+, including tumor size, histologic grade, pT stage. No risk factors were found to be significantly correlated with LVI+ in Table 4. Further logistic regression analysis was applied to determine the risk factors predictive of LN metastasis, including tumor size, histologic grade, pT stage, and LVI. Among the factors examined, LVI (RR, 3.760; 95% CI, 2.087–6.774, p < 0.001) and pT stage (RR, 1.505; 95% CI, 1.272–1.780, p < 0.001) were significantly associated with LN metastasis in Table 5.

4. Discussion

Increasing evidence has confirmed the potential prognostic value of LVI in patients with solid tumors [6–14]. An earlier retrospective study suggested that careful search for vascular invasion in gastric cancer may provide useful information for identifying patients at high risk aged between 23 and 90 years suitable for adjuvant therapy [11]. LVI has been confirmed as an independent prognostic factor in patients aged 32.5–81.1 years with esophageal squamous cell carcinoma [8]. Moreover, LVI has been identified as a strong and independent prognostic factor, and recommended into the TNM staging system for patients aged 17–89 years with primary resected adenocarcinoma of the esophagogastric junction [6].

The majority of previous studies have included patients aged older than 80 or even 85 years. As the average lifespans of men and women in China are 74 and 77 years, respectively, the results would not be as reliable if elderly patients over these age groups are included [15]. If we included patients older than 70 years, they may die because of their own lifespans within 5 years after the surgery, rather than recurrence of metastasis of gastric cancer. Moreover, the 5-YSR of patients is an important index for patients with cancer. Therefore, we only included patients with resected gastric cancer aged younger than 70 years in the present study. However, age selection bias may exist in the current study.

Our two-step multivariate analysis led to the identification of tumor size, LVI, pN stage and adequate or inadequate number of LNs retrieved as independent poor prognostic factors. Tumor sizes ≥4 cm, presence of LVI, higher pN stage, and inadequate number of LNs retrieved were associated with poorer 5-YSR. Considering prognosis, 5-YSR of patients in the LVI+ group was significantly poorer than that of patients without LVI stratified by tumor size and adequate or inadequate number of LNs retrieved. Notably, patients with LVI and inadequate number of LNs retrieved had a 5-YSR of 27.0%, suggesting that both LVI and number of LNs retrieved are prognostic factors for patients with resected gastric cancer.

Considering that pN stage is the most valuable prognostic factor for gastric cancer, we conducted logistic regression analysis of risk factors predictive of LN metastasis [19,20], which revealed a close relationship of metastasis with pT stage and LVI. Importantly, cancers with LVI+

Table 2
Univariate and multivariable analyses of prognostic factors for the entire study population.

Variables	Univariate analysis			Multivariate analysis 1[b]			Multivariate analysis 2[c]		
	n (%)	5-YSR (%)	*p* value	RR	95% CI	*p* value	RR	95% CI	*p* value
Gender			0.121						
Female	156 (26.2)	62.2							
Male	440 (73.8)	54.8							
Age, years			0.627						
< 65	431 (72.3)	56.1							
≥ 65	165 (27.7)	58.2							
Type of anesthesia			0.960						
General anesthesia	499 (83.7)	56.7							
Epidural anesthesia	97 (16.3)	56.7							
Blood loss, ml			0.473						
< 500	554 (93.0)	56.5							
≥ 500	42 (7.0)	59.5							
Tumor size, cm			0.043	1.332	1.062–1.671	0.013	1.319	1.054–1.650	0.015
< 4	169 (28.4)	63.9							
≥ 4	427 (71.6)	53.9							
Reconstruction type			0.176						
Billroth I	486 (81.5)	58.8							
Billroth II	88 (14.8)	46.6							
Roux-Y	22 (3.7)	50.0							
Gastrectomy			0.897						
Total	44 (7.4)	59.1							
Proximal subtotal	59 (9.9)	62.7							
Distal subtotal	493 (82.7)	55.7							
Histologic grade			0.925						
G1	45 (7.6)	62.2							
G2	188 (31.5)	54.3							
G3	327 (54.9)	56.9							
G4	36 (6.0)	61.1							
LVI			< 0.001	1.487	1.122–1.971	0.006	1.489	1.127–1.968	0.005
LVI-	519 (87.1)	59.5							
LVI+	77 (12.9)	37.7							
pT stage [a]			0.372						
pT1	90 (15.1)	61.1							
pT2	143 (24.0)	55.2							
pT3	201 (33.7)	55.7							
pT4a	162 (27.2)	56.8							
pN stage [a]			< 0.001	1.413	1.282–1.558	< 0.001	1.422	1.290–1.566	< 0.001
pN0	273 (45.8)	72.5							
pN1	129 (21.6)	55.0							
pN2	121 (20.3)	38.8							
pN3	73 (12.3)	30.1							
Number of LNs retrieved			0.980	1.304	1.057–1.608	0.013	1.298	1.054–1.599	0.014
Adequate, n ≥ 15	301 (50.5)	56.1							
Inadequate, n < 15	295 (49.5)	57.3							
Recurrence or metastasis			0.208						
Absent	402 (67.4)	58.2							
Present	194 (32.6)	53.6							
Chemotherapy			0.362						
No	517 (86.7)	56.5							
Yes	79 (13.3)	58.2							

n, number of patients; LNs, lymph nodes; RR, relative risk; 95% CI, 95% confidence interval; 5-YSR, five-year overall survival rate (%); LVI-, absence of lymphovascular invasion; LVI+, presence of lymphovascular invasion; G1, well differentiated; G2, moderately differentiated, G3, poorly differentiated, G4, undifferentiated.

[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

[b] All clinicopathologic factors were included in the first multivariate analysis.

[c] Histological grade, pT stage, and chemotherapy were also included in the second multivariate analysis.

had significant high risk of LN metastasis. Our data highlight the importance of LVI for prognosis and its relationship with LN metastasis. LVI may additionally be an effective predictor of LN metastasis. Moreover, pT may be applied as a predictor of LVI. Thus, cancers with higher pT stage may have higher risk of LN metastasis. Patients with higher clinical T stage (cT stage) should therefore be paid more attention and as many LNs (at least 15 if not more) as possible retrieved for accurate staging [17,18], which may be greatly improved with the availability of effective diagnostic methods, such as endoscopic ultrasound (ESU), CT, PET/CT, MRI, and diagnostic staging laparoscopy (DSL) [21–26].

Similarly, we conducted logistic regression analysis to determine the risk factors of LVI. No risk factors were found to be significantly correlated with LVI+. It is our belief that although the current NCCN guidelines for gastric cancer strongly recommend the examination of at least 15 LNs for adequate staging, some patients still have less than 15 LNs retrieved. For pT1, N0 patients (R0 resection), adjuvant therapy is not recommended by the NCCN guidelines. In addition, for pT2, N0 patients, surveillance is also an option. However, for those pT1-2, N0, and LVI+ patients with inadequate LNs retrieved, and who did not receive adjuvant therapy postoperatively, these patients may have a poor survival rate. Therefore, we believe that LVI should be taken into account as an important adjuvant prognostic factor, especially for pT1-2, N0 patients with inadequate LNs retrieved. A previous study also

Table 3
Comparison of prognosis for patients with gastric cancer in the LVI+ and LVI- groups.

Variables	LVI-		LVI+		*p* value
	n (%)	5-YSR (%)	*n* (%)	5-YSR (%)	
For the entire population	519 (100.0)	59.9	77 (100.0)	37.7	< 0.001
Tumor size, cm					
< 4	147 (28.3)	66.7	22 (28.6)	45.5	0.012
≥ 4	372 (71.7)	56.7	55 (71.4)	34.5	< 0.001
pN stage [a]					
pN0	257 (49.5)	73.2	16 (20.8)	62.5	0.162
pN1	114 (22.0)	57.9	15 (19.5)	33.3	0.057
pN2	96 (18.5)	40.6	25 (32.4)	32.0	0.086
pN3	52 (10.0)	30.8	21 (27.3)	28.6	0.275
Number of LNs retrieved					
Adequate, n ≥ 15	261 (50.3)	57.5	40 (51.9)	47.5	0.026
Inadequate, n < 15	258 (49.7)	61.6	37 (48.1)	27.0	< 0.001

n, number of patients; LNs, lymph nodes; RR, relative risk; 95% CI, 95% confidence interval; 5-YSR, five-year overall survival rate (%); LVI-, absence of lymphovascular invasion; LVI+, presence of lymphovascular invasion.
[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

Table 4
Logistic regression analysis of risk factors predicting the presence of LVI.

Variables	RR	95% CI	*p* value
Tumor size	0.996	0.585–1.694	0.988
Histologic grade	1.227	0.867–1.735	0.248
pT stage[a]	1.086	0.855–1.379	0.498

RR, relative risk; 95% CI, 95% confidence interval.
[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

Table 5
Logistic regression analysis of risk factors predicting LN metastasis.

Variables	RR	95% CI	*p* value
Tumor size	1.075	0.740–1.561	0.705
Histologic grade	1.150	0.909–1.455	0.242
pT stage[a]	1.505	1.272–1.780	< 0.001
LVI	3.760	2.087–6.774	< 0.001

n, number; RR, relative risk; 95% CI, 95% confidence interval; LN, lymph node; LVI, lymphovascular invasion.
[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

suggested that adjuvant therapy should be considered for LVI+ patients [27]. Therefore, pathology reports in the future should include assessment of LVI. We recommend that LVI should be taken into account as an important adjuvant prognostic factor, especially for pN0 patients with inadequate LNs retrieved. The key for an adequate prognostic assessment of gastric cancer is an adequate lymph node yield. Though the NCCN guidelines for gastric cancer strongly recommend the examination of at least 15 LNs for adequate staging, more lymph node dissection will be better. It is quite obvious that a LVI+ case with inadequate lymph node yield may be misdiagnosed as a N0, being instead a N1, considering the skip metastases issue and the aggressivity of histologic pattern. Therefore, this could lead to undertreatment. A dissection of less than 15 lymph nodes is an inadequate treatment for gastric cancer, which may be not an ideal surgery. Importantly, the LV paten can be an additional help to stratify the risk of recurrence. As far as we are concerned that a retrospective study may be liable for biases; therefore, further multicenter, randomized controlled trials, especially containing postoperative pathology reports as subject, are required.

Overall, our results indicate that LVI is an independent prognostic factor predicting LN metastasis and a strongly independent predictor of survival for patients with resected gastric cancer, specially pN0 cases with inadequate LNs retrieved. In addition, the maximum number of LNs possible should be retrieved for optimal staging, especially for patients with higher cT stage. However, the results of the current study need to be interpreted with caution and further multicenter, randomized controlled trials are required to validate our findings.

Ethical approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with Helsinki Declaration of 1964 and later versions.

Sources of funding

This study was funded by the Natural Science Foundation of Liaoning Province (No: 201602817).

Author contribution

CDZ, FLN and DQD conceived of the study. CDZ and FLN collected data. CDZ and FLN provided data analysis. All authors contributed to writing, reviewing, or revising the paper. CDZ, FLN and DQD submitted the final manuscript and all authors read and approved the final manuscript. All authors are grateful to all the previous study authors and study participants.

Conflict-of-interest

The authors declare that they have no conflict of interest.

Research registration unique identifying number

UIN is researchregistry3313.

Guarantor

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References

[1] R.L. Siegel, K.D. Miller, A. Jemal A, Cancer statistics, 2017, CA Cancer J. Clin. 67 (2017) 7–30.
[2] W.B. Goggins, G.K. Wong, Poor survival for US pacific islander cancer patients: evidence from the surveillance, epidemiology, and end results database: 1991 to 2004, J. Clin. Oncol. 25 (2007) 5738–5741.
[3] K. Washington, 7th edition of the AJCC cancer staging manual: stomach, Ann. Surg Oncol. 17 (2010) 3077–3079.
[4] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, Global cancer statistics, 2002, CA Cancer J. Clin. 55 (2005) 74–108.
[5] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, et al., Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2016) 115–132.
[6] B.H. von Rahden, H.J. Stein, M. Feith, K. Becker, J.R. Siewert, Lymphatic vessel invasion as a prognostic factor in patients with primary resected adenocarcinomas of the esophagogastric junction, J. Clin. Oncol. 23 (2005) 874–879.
[7] M. Sarbia, R. Porschen, F. Borchard, O. Horstmann, R. Willers, et al., Incidence and prognostic significance of vascular and neural invasion in squamous cell carcinomas of the esophagus, Int. J. Canc. 61 (1995) 333–336.
[8] B.L. Brücher, H.J. Stein, M. Werner, J.R. Siewert, Lymphatic vessel invasion is an independent prognostic factor in patients with a primary resected tumor with esophageal squamous cell carcinoma, Cancer 92 (2001) 2228–2233.
[9] S.M. Lagarde, A.W. Phillips, M. Navidi, B. Disep, A. Immanuel, et al., The presence of lymphovascular and perineural infiltration after neoadjuvant therapy and oesophagectomy identifies patients at high risk for recurrence, Br. J. Canc. 113 (2015) 1427–1433.
[10] C.D. Zhang, J.N. Wang, B.Q. Sui, Y.J. Zeng, J.Q. Chen, et al., Prognostic and

predictive model for stage II colon cancer patients with nonemergent surgery: who should receive adjuvant chemotherapy? Medicine (Baltim.) 95 (2016) e2190.
[11] H.E. Gabbert, S. Meier, C.D. Gerharz, G. Hommel, Incidence and prognostic significance of vascular invasion in 529 gastric-cancer patients, Int. J. Canc. 49 (1991) 203–207.
[12] B.J. Dicken, K. Graham, S.M. Hamilton, S. Andrews, R. Lai, et al., Lymphovascular invasion is associated with poor survival in gastric cancer: an application of gene-expression and tissue array techniques, Ann. Surg. 243 (2006) 64–73.
[13] P. Li, H.Q. He, C.M. Zhu, Y.H. Ling, W.M. Hu, et al., The prognostic significance of lymphovascular invasion in patients with resectable gastric cancer: a large retrospective study from Southern China, BMC Canc. 15 (2015) 370.
[14] T. Yamada, H. Sugiyama, D. Ochi, D. Akutsu, H. Suzuki, et al., Risk factors for submucosal and lymphovascular invasion in gastric cancer looking indicative for endoscopic submucosal dissection, Gastric Cancer 17 (2014) 692–696.
[15] L. Zong, M. Abe, Y. Seto, J. Ji, Randomized controlled trial of laparoscopic versus open D2 distal gastrectomy for advanced gastric cancer: how should we define the age of included patents? J. Clin. Oncol. (2016), http://dx.doi.org/10.1200/JCO.2016.68.2500 pii: JCO682500.
[16] R.A. Agha, M.R. Borrelli, M. Vella-Baldacchino, R. Thavayogan, D.P. OrgillSTROCSS Group, The STROCSS statement: strengthening the reporting of cohort studies in surgery, Int. J. Surg. 46 (2017) 198–202.
[17] R.E. Schwarz, D.D. Smith, Clinical impact of lymphadenectomy extent in resectable gastric cancer of advanced stage, Ann. Surg Oncol. 14 (2007) 317–328.
[18] I. Songun, H. Putter, E.M. Kranenbarg, M. Sasako, C.J. van de Velde, Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial, Lancet Oncol. 11 (2010) 439–449.
[19] M. Degiuli, M. Sasako, A. Ponti, F. Calvo, Survival results of a multicentre phase II study to evaluate D2 gastrectomy for gastric cancer, Br. J. Canc. 90 (2004) 1727–1732.
[20] G. de Manzoni, G. Verlato, F. Roviello, F. Morgagni, A. Di Leo A, et al., The new TNM classification of lymph node metastasis minimises stage migration problems in gastric cancer patients, Br. J. Canc. 87 (2002) 171–174.
[21] T. Ichikura, S. Tomimatsu, K. Uefuji, M. Kimura, T. Uchida, D. Morita, et al., Evaluation of the New American Joint Committee on Cancer/International Union against cancer classification of lymph node metastasis from gastric carcinoma in comparison with the Japanese classification, Cancer 86 (1999) 553–558.
[22] E.K. Abdalla, P.W. Pisters, Staging and preoperative evaluation of upper gastrointestinal malignancies, Semin. Oncol. 31 (2004) 513–529.
[23] R.M. Kwee, T.C. Kwee, Imaging in local staging of gastric cancer: a systematic review, J. Clin. Oncol. 25 (2007) 2107–2116.
[24] W.A. Weber, K. Ott, Imaging of esophageal and gastric cancer, Semin. Oncol. 31 (2004) 530–541.
[25] D. Bentrem, H. Gerdes, L. Tang, M. Brennan, D. Coit, Clinical correlation of endoscopic ultrasonography with pathologic stage and outcome in patients undergoing curative resection for gastric cancer, Ann. Surg Oncol. 14 (2007) 1853–1859.
[26] T. Irino, T. Sano, N. Hiki, M. Ohashi, S. Nunobe, et al., Diagnostic staging laparoscopy in gastric cancer: a prospective cohort at a cancer institute in Japan, Surg. Endosc. 32 (2018) 268–275.
[27] C. Du, Y. Zhou, K. Huang, G. Zhao, H. Fu, et al., Defining a high-risk subgroup of pathological T2N0 gastric cancer by prognostic risk stratification for adjuvant therapy, J. Gastrointest. Surg. 15 (2011) 2153–2158.

International Journal of Surgery 56 (2018) 31–43

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.elsevier.com/locate/ijsu

Review

Reevaluation of laparoscopic versus open distal gastrectomy for early gastric cancer in Asia: A meta-analysis of randomized controlled trials

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ARTICLE INFO

Keywords:
Laparoscopy
Early gastric cancer
Gastrectomy
Meta-analysis

ABSTRACT

Background: The benefits and risks of laparoscopic distal gastrectomy (LADG) are not yet sufficiently clear for acceptance as a standard treatment of early gastric cancer. Previous meta-analyses were not powered to reach definitive conclusions.

Materials and Methods: Randomized controlled trials comparing LADG with open distal gastrectomy (ODG) for early gastric cancer in Asia and published between January 1994 and January 2018 were retrieved from PubMed, Embase, the Cochrane Library, and Google Scholar. Patient characteristics, oncological safety and efficacy, and surgical safety were evaluated following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Grading of Recommendations Assessment, Development and Evaluation guidelines (GRADE) guidelines. Trial Sequential Analysis (TSA) reduced random error and reinforced the reliability and strength of evidence.

Results: Eight trials including 2666 participants were selected. LADG benefits were an 11.6 cm shorter incision (95% CI: −13.31 to −9.88 cm; $P < 0.0001$), 103.81 ml less blood loss (95% CI: −133.68 to −73.94; $P < 0.0001$), 1.73 times less analgesic use (95% CI: −2.21 to −1.24; $P < 0.0001$), 0.51 days shorter time to first flatus (95% CI: −0.88 to −0.15 days; $P = 0.006$), lower risk of wound dehiscence (RR = 0.24, 95% CI: 0.08–0.78; $P = 0.02$), lower risk of surgical adverse events (RR = 0.69, 95% CI: 0.53–0.91; $P = 0.008$), and lower risk of respiratory complications (RR = 0.40; 95% CI: 0.20–0.79; $P = 0.009$) than ODG. LADG had 2.22 fewer resected lymph nodes (95% CI: −4.33 to −0.12; $P = 0.04$) and 76.61 min longer procedures (76.61 min, 95% CI: 57.74–95.47 min; $P < 0.0001$).

Conclusions: In Asian patients, LADG had similar mortality and oncological safety, better surgical safety, less operative morbidity, less trauma, and faster recovery than ODG. It has a high role to play in node-negative cases due to better short-term outcomes but less nodal harvest. It is a recommended alternative treatment for experienced surgeons in high-volume centers.

1. Introduction

Gastric cancer is a public health concern worldwide, and especially in Asia [1–3]. Laparoscopic distal gastrectomy (LADG) for gastric cancer was introduced by Kitano et al., in 1994 [4]. Since then, interest in this minimally invasive surgical procedure for the treatment of patients with gastric cancer has been increasing. Its perceived benefits include less trauma, operative blood loss, morbidity, and postoperative pain, and accelerated recovery than open distal gastrectomy (ODG); simultaneously, its perceived risks are related to its complexity and a long learning curve that can prolong the procedure, uncertain surgical safety, inadequate lymph node clearance, and incomplete resection [5–14]. LADG is not yet a standard technique for resection of gastric cancer. Further study is needed before it can be recommended.

LADG is technically complex compared with ODG, and the resulting need for adequate training and experience is one reason that this technique has not yet become accepted worldwide as an alternative gastric cancer treatment. In addition, oncological and surgical safety need to be guaranteed before adoption. It may take longer for LADG to be routinely used to treat patients with advanced than early gastric cancer. LADG is more frequently used to treat patients in Asian countries such as Japan and South Korea, where screening programs have resulted in higher rates of early diagnosis than in other counties [1,15–17]. As early gastric cancer is highly curable, close attention

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https://doi.org/10.1016/j.ijsu.2018.05.733
Received 1 May 2018; Received in revised form 20 May 2018; Accepted 27 May 2018
Available online 31 May 2018
1743-9191/

should be paid to surgical safety.

The results of randomized controlled trials (RCTs) [5–14] and non-RCTs [18–23] comparing LADG and ODG in patients with early gastric cancer are inconsistent, and previous meta-analyses of RCTs have lacked statistical power [24–32]. Potential bias is likely to be greater for non-RCTs because the quality of evidence is lower than that of RCTs. Consequently, the results of meta-analyses including non-RCTs should be interpreted with caution [33]. The two most recent meta-analyses that included only RCTs included 390 and 732 patients, respectively [31,32]. They lacked adequate power to reach definitive conclusions and may have included false positive errors. Three additional RCTs including 2359 patients have been published and will strengthen the current evidence [34–36]. Before a recommendation for routine clinical use of LADG for patients with early gastric cancer can be made, a high level of evidence is required.

This meta-analysis of the latest available evidence from RCTs re-evaluated the safety and efficacy of LADG compared with ODG. It targeted early gastric cancer because its low probability of lymph node metastasis. The quality of the evidence was evaluated by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool [37,38], and Trial Sequential Analysis (TSA) was used to determine whether the current evidence was sufficient and conclusive [39–43].

2. Materials and methods

The meta-analysis included the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, http://www.prisma-statement.org/) checklist [44]. No registered protocol was applied in the current meta-analysis. The Cochrane Handbook for Systematic Review of Interventions was applied to perform the meta-analysis [33]. The meta-analysis data is available on any reasonable request.

2.1. Search strategies

PubMed, Embase, the Cochrane Library, and Google Scholar were searched for articles published from January 1994 to January 2018 [4], without language restriction. Other non-English language articles were screened using Google Translate (https://translate.google.cn/). The search used Medical Subject Headings (MeSH) and keywords including MeSH “Laparoscopy”, and keywords “laparoscopic”, and “laparoscopy-assisted” and MeSH “Stomach Neoplasms” and keywords “gastric cancer” and “stomach cancer”. Additional searches were performed in the ClinicalTrials.gov registry (www.clinicaltrials.gov) and the reference lists of retrieved studies to identify other potentially eligible articles.

2.2. Selection criteria

RCTs were eligible for inclusion if they included patients with early gastric cancer requiring distal gastrectomy and not suitable for endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD). The eligible interventions were LADG or laparoscopy-assisted distal gastrectomy and comparison with ODG. Eligible studies reported more than one of the following outcomes: procedure-related, postoperative, prognosis, and adverse events.

2.3. Screening and extraction

Two authors independently carried out the initial screening and removed duplicates. References in the included RCTs were screened for eligible articles. All discrepancies were resolved by discussion. If two or more articles were published by the same team from the same institute and contained the same or some of the same participants, only the most detailed article was included. The first author, year of publication, country, number of participants, mean age, treatment, study design, follow-up, reconstruction type, lymph node dissection, surgeon experience, and outcomes data were extracted from each included study. The patient characteristics included age, body mass index (BMI), tumor size, procedure time, length of incision, blood loss, blood transfusion volume, reoperation, operation-related deaths, analgesic use, time to first flatus, time to first water/food intake, postoperative hospital stays, number of lymph nodes retrieved, positive lymph nodes, recurrence, wound infection, wound dehiscence, anastomotic stenosis, postoperative bleeding, delayed gastric emptying, intra-abdominal abscess/fluid collection, pancreatic complications, chyle leakage, overall surgical adverse events, respiratory complications, and surgeon experience. Accordingly, recurrence was recorded until the end of the follow-up periods [5–9,34–36].

2.4. Risk of bias assessment

Two authors independently evaluated the risk of bias for each RCT using the Cochrane Risk of Bias tool [33,45]. The risk of bias in random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and others was scored as high, low or unclear [33,45]. Blinding of participants and personnel was difficult to perform in these RCTs, but the outcomes may be less prone to be influenced by lack of blinding. Any disagreements were resolved by discussion.

2.5. Quality of evidence

Two authors independently assessed the quality of evidence provided by the study outcomes using the GRADE tool (version 3.2, GRADEpro, https://gradepro.org/). The risk of bias, inconsistency, indirectness, imprecision, and other considerations were included in the evaluation, and were scored as very low, low, moderate, and high quality [37,38]. The quality of evidence for operation-related deaths, lymph nodes retrieved, recurrence, reoperation, overall surgical adverse events, and time to first flatus, and other outcomes were evaluated.

2.6. Statistical analysis

The statistical analysis was performed using Review Manager 5.3.5 (Nordic Cochrane Centre), which is recommended by both the PRISMA statement and the Cochrane Library [33,44], and included intention-to-treat populations. Dichotomous variables were assessed by risk ratios (RRs) with 95% confidence intervals (CIs). Continuous variables were assessed by mean differences (MDs) with 95% CIs. A P value < 0.05 was considered statistically significant. A random-effects model was applied to account for methodological or clinical heterogeneity. Methodological heterogeneity among the RCTs was quantified by I^2 and P values, and $I^2 > 50\%$ or $P < 0.10$ indicated significant methodological heterogeneity [33,37]. Publication bias was assessed in funnel plots [44,46]. Sample means and standard deviation (SD) were estimated from sample size, median, range and/or interquartile range, if means and SD were not directly reported in the RCTs [47,48].

2.7.

TSA can reduce false positive (type I) errors by combining the required information size (RIS) and adjusted threshold for statistical significance [39–43]. As early gastric cancer is a highly curable disease; more attention was paid to surgical than to oncological safety in this meta-analysis. Thus, TSA was conducted to estimate the RIS of the overall surgical adverse events using $\alpha = 0.05$, and $\beta = 0.20$ (a power of 80%). The conclusion was sufficient and credible if the cumulative z curve crossed either the trial sequential monitoring boundary or the RIS, with no requirement for further trials [39–43]. TSA software version 0.9.5.10 beta (http://www.ctu.dk/tsa) was used for this analysis.

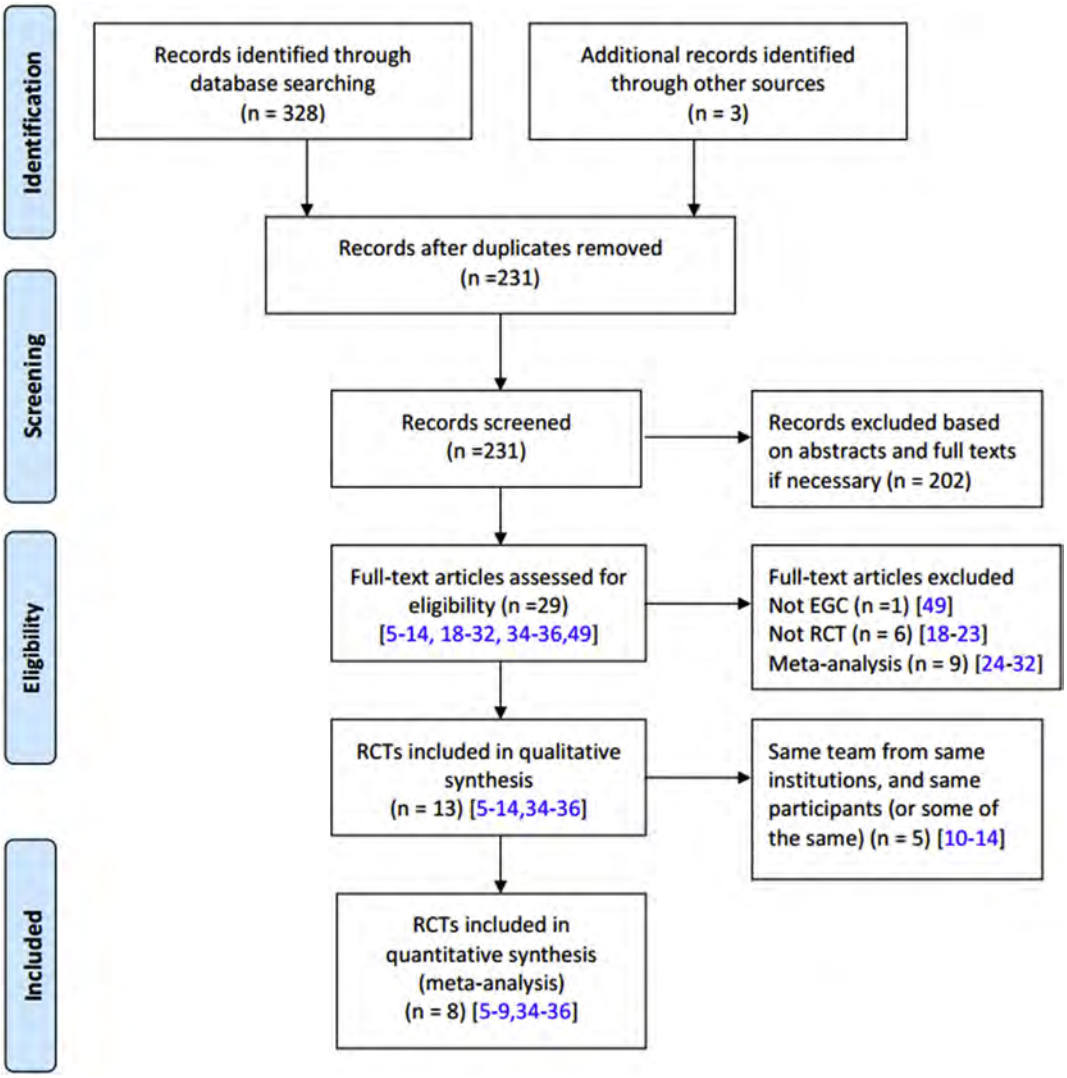

Fig. 1. Flow diagram of study selection.

3. Results

3.1. Trial selection

A flow diagram of the trial selection process and reasons for exclusion is shown (Fig. 1). A total of 331 articles were retrieved, and after removing duplicates and screening the abstract, and full text if necessary, the full text of the 29 remaining articles were screened for eligibility [5–14,18–32,34–36,49]. One article not associated with early gastric cancer [49], six that were not RCTs [18–23], and nine meta-analyses were excluded [24–32]. Of the remaining 13 RCTs [5–14,34–36], five were excluded because they were thought to be published by the same team from the same institute, and contained the same or some of the same, participants [10–14]. Eight RCTs were eventually included in the meta-analysis [5–9,34–36]. Necessary information was still obtained from the five excluded trials [10–14].

3.2. Trial characteristics

Five trials were from Japan and three were from South Korea. They were published between 2002 and 2017, the sample sizes ranged from 28 to 1,384, and a total of 2666 patients were included. The mean age of LADG patients ranged from 56 to 63.2 years; that of ODG patients was 54.5–63.5 years. The median follow-up ranged from 14 to 74.3 months. The reconstruction types in three trials included B-I, B-II, and Roux-en-Y [5,34,35], and B-1 was the only reconstruction performed in four trials [6–9]. The lymph node dissection types included D1, modified D2 lymphadenectomy (D1 +), and D2 lymphadenectomy. The surgeon experience, study design, and outcomes are summarized in Table 1.

3.3. Risk of bias assessment

The risk of bias evaluation of the included RCTs is summarized in Table 2 and Fig. 2. Allocation concealment risk was unclear in five trials [6–9,35], high risk in two [5,34], and low risk in only one trial [36]. Blinding of participants and personnel is difficult to perform in clinical trials, and bias was at high risk in six [5,7,8,34–36], unclear risk in one [9], and at low risk in only one [6]. All trials were at low risk of bias in generation of random number sequences, blinding of outcome assessment, incomplete outcome data, selective reporting and others [5–9,34–36].

3.4. Patient baseline characteristics

The patient baseline characteristics are summarized in Table 3, Fig. 3, and Figs. S1–S3. There were no significant differences in age (MD −0.28 years, 95% CI, −1.75 to 1.19 years, $P = 0.71$; Fig. S1)

Table 1
Characteristics of included randomized controlled trials.

Included Trials	Participants Mean age (years)	Treatments (Number)	Study Design	Follow UP (months)	Reconstruction Type	Lymph Node Dissection	Surgeon Experience	Outcomes
Katai et al., 2017 [34] (Japan)	EGC patients in cT1N0-1, cT2N0 stages. 63.2 vs 63.5	LADG (n = 457) ODG (n = 455)	Multi-center RCT	60.0	B-I B-II Roux-en-Y Gastro-gastro	D1 D1+ D2	LADG: experience of at least 30 of both LADG and ODG operations; ODG: experience of at least 60 ODG operations.	1-9, 11, 14, 17–29.
Kim et al., 2016 [35] (SK)	EGC patients in cT1N0-1, cT2aN0 stages. 56.8 vs 57.6	LADG (n = 686) ODG (n = 698)	Multi-center Prospective RCT	60.0	B-I B-II Roux-en-Y	D1+β D2	Experience of at least 50 each of LADG and ODG. They established a standardized protocol of the procedure.	1-4, 6–9, 13–15, 17–22, 24–29.
Yamashita et al., 2016 [36] (Japan)	EGC patients in cT1 stage. 58.0 vs 61.0	LADG (n = 31) ODG (n = 32)	Single-center RCT	Median: 63.0	Standardized according to the JCGC 2nd English edition.	Standardized according to the JCGC 2nd English edition.	LADG: experience of more than 100 LADG operations; ODG: experience of more than 500 ODG operations.	1-4, 6, 8–11, 13–16, 19, 21, 23, 24, 27–28.
Kim et al., 2013 [5] (SK)	EGC patients in cT1N0-1 stages. 56.7 vs 54.5	LADG (n = 82) ODG (n = 82)	Prospective, RCT	Median: 74.3	B-I B-II Roux-en-Y	D2	A single surgeon with no details of experience.	1-7, 9, 11–14, 16, 17, 21, 23, 24, 27, 29.
Takiguchi et al., 2013 [6] (Japan)	EGC patients in cT1N0-1 stages. 61.5 vs 62.5	LADG (n = 20) ODG (n = 20)	Prospective, RCT	At least 60.0	B-I	D1 D2	A single surgeon well trained in both LADG and ODG operations.	1-7, 9, 14, 29.
Hayashi et al., 2005 [7] (Japan)	EGC patients in cT1 stage. 56.0 vs 62.0	LADG (n = 14) ODG (n = 14)	Prospective, RCT	Median: 39 vs 45	B-I	D2	Both LADG and ODG were performed by a single surgical team that had wide experience with open and laparoscopic procedures.	1, 2, 4, 6, 7, 9–16, 19, 20, 27–29.
Lee et al., 2005 [8] (SK)	EGC patients in cT1 stage. 56.6 vs 59.5	LADG (n = 24) ODG (n = 23)	Prospective, RCT	Median: 14.0	B-I	D2	NM.	1, 4–6, 9–17, 19, 28, 29.
Seigo et al., 2002 [9] (Japan)	EGC patients in cT1 stage. 63.2 vs 60.1	LADG (n = 14) ODG (n = 14)	RCT	Median: 26.0	B-I	NM	An experienced surgeon with the same surgical team.	1, 3–6, 9–16, 23, 25, 27–29.

PICOS, Participants-Interventions-Comparisons-Outcomes-Study design; LADG, laparoscopic-assisted distal gastrectomy; ODG, open distal gastrectomy; SK, South Korea; EGC, early gastric cancer; vs, versus; cTNM, clinical TNM staging; NM, not mentioned; JCGC, Japanese Classification of Gastric Cancer; B-I, billroth I; B-II, billroth II.
Outcomes: 1. age; 2. BMI; 3. tumor size; 4. operation time; 5. length of incision; 6. blood loss; 7. blood transfusion volume; 8. reoperation; 9. operation-related deaths; 10. analgesic use; 11. time to first flatus; 12. time to first intake of water/food; 13. duration of postoperative hospital stays; 14. lymph nodes retrieved; 15. positive lymph nodes; 16. recurrence; 17. wound infection; 18. wound dehiscence; 19. anastomotic stenosis; 20. anastomotic stenosis, 21. postoperative bleeding; 22. postoperative bleeding; 23. delayed gastric emptying; 24. intraabdominal abscess/fluid collection; 25. pancreatic complications; 26. chyle leakage; 27. overall surgical adverse events; 28. respiratory complications; 29. surgeon experience.

Table 2
Risk of bias in the included trials assessed with the Cochrane Risk of Bias tool.

Included Trials	Random Sequence Generation[a]	Allocation Concealment[b]	Blinding of Participants and Personnel[c]	Blinding of Outcome Assessment[d]	Incomplete Outcome Data[e,h]	Selective Reporting[f]	Other Bias[g]
Katai 2017 [34]	Low risk	High risk	High risk	Low risk	Low risk	Low risk	Low risk
Kim 2016 [35]	Low risk	Unclear risk	High risk	Low risk	Low risk	Low risk	Low risk
Yamashita 2016 [36]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Kim 2013 [5]	Low risk	High risk	High risk	Low risk	Low risk	Low risk	Low risk
Takiguchi 2013 [6]	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hayashi 2005 [7]	Low risk	Unclear risk	High risk	Low risk	Low risk	Low risk	Low risk
Lee 2005 [8]	Low risk	Unclear risk	High risk	Low risk	Low risk	Low risk	Low risk
Seigo 2002 [9]	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk

RCTs, randomized controlled trails.
[a] Selection bias.
[b] Selection bias.
[c] Performance bias.
[d] Detection bias.
[e] Attrition bias.
[f] Reporting bias.
[g] Other source of bias.
[h] Blinding of participants and personnel was difficult in these RCTs, but the outcomes may be less prone to be influenced by lack of blinding.

[5–9,34–36], BMI (MD −0.02, −0.23 to 0.20, $P = 0.88$; Fig. S2) [5–7,34–36], or tumor size (MD −0.13 cm, −0.40 to 0.14 cm, $P = 0.33$; Fig. S3) [5,6,9,34–36] in the LADG and ODG groups.

3.5. Procedure-related outcomes

The procedure-related outcomes are summarized in Table 3, Fig. 4, and Figs. S4–S9. The procedure duration was 76.61 min longer (95% CI: 57.74–95.47 min, $P < 0.0001$; Fig. S4) [5–9,34–36], the incision was 11.60 cm shorter (95% CI: −13.31 to −9.88 cm, $P < 0.0001$; Fig. S5) [5,6,8,9,34], and the blood loss was 103.81 ml less (95% CI: −133.68 to −73.94 ml $P < 0.0001$; Fig. S6) [8–12,34–36] with LADG than with ODG. There were no differences between LADG and ODG in blood transfusion volumes (RR 0.90; 95% CI: 0.35–2.33, $P = 0.83$; Fig. S7) [5–7,34,35], reoperation (RR 0.85, (95% CI: 0.38–1.93, $P = 0.70$; Fig. S8) [34–36], or operation-related deaths (RR 2.03, 95% CI: 0.37–11.07; $P = 0.41$; Fig. S9) [5–9,34–36].

3.6. Postoperative outcomes

The postoperative outcomes are summarized in Table 3, Fig. 5, and Figs. S10–S13. Use of analgesics was 1.73 times less (95% CI: −2.21 to −1.24, $P < 0.0001$; Fig. S10) [7–9,36], and time to first flatus was 0.51 days shorter (95% CI −0.88 to −0.15 days $P = 0.006$; Fig. S11) [5,7–9,34,36] with LADG than with ODG. The differences between LADG and ODG in time to first intake water/food (−0.45 days; 95% CI: −1.40 to 0.50 days, $P = 0.35$; Fig. S12) [5,7–9], and duration of postoperative hospital stay (−1.02 days, 95% CI: −2.06 to 0.01 days, $P = 0.05$; Fig. S13) [5,7–9,35,36] were not significant.

3.7. Prognosis outcome

The prognosis outcomes are summarized in Table 3, Fig. 6, and Figs. S14–S16. LADG patients had 2.22 fewer resected lymph nodes than ODG patients (95% CI: −4.33 to −0.12; $P = 0.04$, Fig. S14) [5–9,34–36]. There were no significant differences in positive lymph nodes (RR 0.93, 95% CI: 0.74–1.18; P = 0.57; Fig. S15) [7–9,35,36], or recurrence (RR 0.50, 95% CI: 0.05–5.41; P = 0.57; Fig. S16) [5,7–9,36].

3.8. Adverse event outcomes

The adverse event outcomes are summarized in Table 3, Fig. 7, and Figs. S17–S28. Wound dehiscence (RR 0.24, 95% CI: 0.08–0.78; $P = 0.02$; Fig. S18) [34,35] and risk of respiratory complications (RR 0.40, 95% CI: 0.20–0.79; $P = 0.009$; Fig. S28) were lower with LADG than with ODG [7–9,34–36]. Seven RCTs including 2626 participants reported the overall occurrence of surgical adverse events [5,7–9,34–36]. LADG had a significant lower risk of surgical adverse events than ODG (RR 0.69, 95% CI: 0.53–0.91; $P = 0.008$; Fig. S27) [5,7–9,34–36]. The TSA cumulative z curve crossed the trial sequential monitoring boundary for benefit in LADG, indicating that the evidence is sufficient and conclusive (Fig. 8). TSA thus indicated that early gastric cancer patients would benefit from LADG by having fewer surgical adverse events compared with ODG. Additional RCTs might not be required and might be unlikely to change the current conclusion.

There were no significant differences between LADG and ODG in infection (RR 1.11, 95% CI: 0.47–2.60; $P = 0.81$; Fig. S17) [5,8,34,35], anastomotic stenosis (RR 1.00, 95% CI: 0.28–3.63; $P = 1.00$; Fig. S19) [7,8,34–36], anastomotic leakage (RR 0.63, 95% CI: 0.24–1.65, $P = 0.34$; Fig. S20) [7,34,35], postoperative bleeding (RR 0.69, 95% CI: 0.40–1.19; $P = 0.18$; Fig. S21) [5,34–36], postoperative obstruction/ileus (RR 0.82, 95% CI 0.44–1.55; $P = 0.54$; Fig. S22) [34,35], delayed gastric emptying (RR 0.46, 95% CI: 0.16–1.30; $P = 0.14$; Fig. S23) [5,9,34,36], intra-abdominal abscess/fluid collection (RR 0.71, 95% CI: 0.36–1.39; $P = 0.31$; Fig. S24) [5,34–36], pancreatic complications (RR 1.67, 95% CI: 0.40–6.90; $P = 0.48$; Fig. S25) [9,34,35], or chyle leakage (RR 0.34, 95% CI: 0.01–8.31, $P = 0.51$; Fig. S26) [34,35].

3.9. Learning curve and surgeon experience

Six RCTs reported surgeon experience [6,7,9,34–36], three gave the minimum experience, which ranged from 30 to 100 cases for LADG, and 50 to 500 cases for ODG [34–36]. Three noted that the surgeons or surgical team included “a single surgeon well trained in both LADG and ODG” [6], “a single surgical team that had wide experience with open and laparoscopic procedures” [7], and “an experienced surgeon with the same surgical team” [9].

3.10. GRADE working group scores of evidence and publication bias

GRADE working group evidence scores for the RCT outcomes are summarized in Table 4. The level of evidence was low for operation-related deaths [5–9,34–36], lymph nodes retrieved [5–9,34–36], and overall survival-adverse events [5,7–9,34–36]; and very low for recurrence [5,7–9,36], reoperation [34–36], and time to first flatus [5,7–9,34,36]. The funnel plots showing publication bias are shown in Fig. 9. Publication bias was indicated by funnel plot asymmetry, the

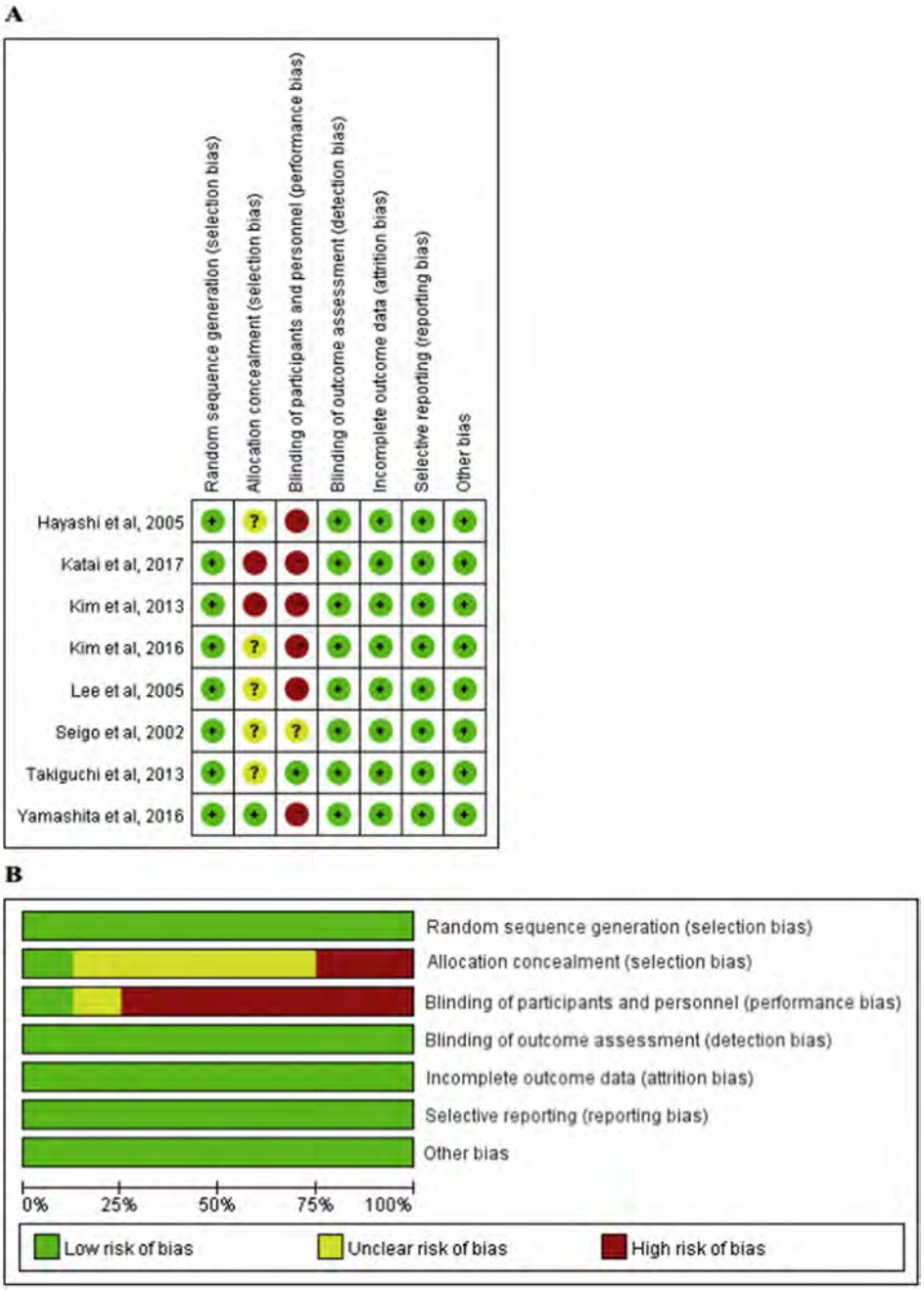

Fig. 2. A Bias risk summary for each element in all included trials; **B** Bias risk for each element as a percentage across all included trials.

absence of RCTs with negative results, and a total of less than nine included RCTs [33,46].

4. Discussion

Early gastric cancer is considered highly curable because of a low probability of lymph node metastasis. LADG cannot be recommended for routine treatment of patients with gastric cancer if its superiority to ODG is not guaranteed. This technically complex and time-consuming procedure should be initially evaluated in early stage disease, and a considerably longer evaluation may be required before LADG is routinely used to advanced gastric cancer. Existing screening programs may account for the higher incidence and diagnosis of early gastric cancer in Japan and South Korea than in western countries [1,15–17]. Furthermore, surgeons in Asian countries, especially Japan and South Korea, might be more experienced in the surgical treatment of gastric cancer than those in western countries. Also, patients in Asia have average lower BMIs than western patients. Previous studies found that a high BMI did not increase the incidence of surgical complications of LADG compared with ODG [50,51]. However, patients with higher BMIs will increase the technical complexity of LADG, with risk of less nodal harvest, prolonged procedure time, and increased postoperative blood loss. Accordingly, this meta-analysis limited the comparison of LADG with ODG in early gastric cancer.

Before evaluation of the oncological safety and effectiveness of LADG, its surgical safety should be guaranteed. This meta-analysis found LADG took longer time than ODG. LADG with lymphadenectomy is a relatively new, time-consuming procedure that is technically complex, but surgeons can overcome those issues through continuous training. Compared with ODG, LADG offers significant benefits of less

Table 3
Main results of meta-analyses including all the outcomes.

Variable	No. of Trials	No. Participants		Effect Estimate RR/MD (95% CI)	*P* Value
		LADG	Total		
Patient baseline characteristics					
Age (years) [5–9,34–36]	8	1328	2666	MD, −0.28 (−1.75, 1.19)	0.71
BMI [5–7,34–36]	6	1290	2591	MD, −0.02 (−0.23, 0.20)	0.88
Tumor size (cm) [5,6,9,34–36]	6	1290	2591	MD, −0.13 (−0.40, 0.14)	0.33
Procedure-related outcomes					
Operation time (mins) [5–9,34–36]	8	1328	2666	MD, 76.61 (57.74, 95.47)	< 0.0001*
Length of incision (cm) [5,6,8,9,34]	5	597	1191	MD, −11.60 (−13.31, −9.88)	< 0.0001*
Blood loss (ml) [5–9,34–36]	8	1328	2666	MD, −103.81 (−133.68, −73.94)	< 0.0001*
Blood transfusion volume [5–7,34,35]	5	1259	2528	RR, 0.90 (0.35, 2.33)	0.83
Reoperation [34–36]	3	1174	2359	RR, 0.85 (0.38, 1.93)	0.70
Operation-related deaths [5–9,34–36]	8	1328	2666	RR, 2.03 (0.37, 11.07)	0.41
Postoperative outcomes					
Analgesic use [7–9,36]	4	83	166	MD, −1.73 (−2.21, −1.24)	< 0.0001*
Time to first flatus (days) [5,7–9,34,36]	6	622	1242	MD, −0.51 (−0.88, −0.15)	0.006*
Time to first intake of water/food (days) [5,7–9]	4	134	267	MD, −0.45 (−1.40, 0.50)	0.35
Duration of postoperative hospital stays (days) [5,7–9,35,36]	6	851	1714	MD, −1.02 (−2.06, 0.01)	0.05
Prognosis outcomes					
Lymph nodes retrieved [5–9,34–36]	8	1328	2666	MD, −2.22 (−4.33, −0.12)	0.04*
Positive lymph nodes [7–9,35,36]	5	769	1550	RR, 0.93 (0.74, 1.18)	0.57
Recurrence [5,7–9,36]	5	165	330	RR, 0.50 (0.05, 5.41)	0.57
Adverse event outcomes					
Wound infection [5,8,34,35]	4	1249	2507	RR, 1.11 (0.47, 2.60)	0.81
Wound dehiscence [34,35]	2	1143	2296	RR, 0.24 (0.08, 0.78)	0.02*
Anastomotic stenosis [7,8,34–36]	5	1212	2434	RR, 1.00 (0.28, 3.63)	1.00
Anastomotic leakage [7,34,35]	3	1157	2324	RR, 0.63 (0.24, 1.65)	0.34
Postoperative bleeding [5,34–36],[a]	4	1256	2523	RR, 0.69 (0.40, 1.19)	0.18
Postoperative obstruction/ileus [34,35]	2	1143	2296	RR, 0.82 (0.44, 1.55)	0.54
Delayed gastric emptying [5,9,34,36]	4	584	1167	RR, 0.46 (0.16, 1.30)	0.14
Intraabdominal abscess/fluid collection [5,34–36]	4	1256	2523	RR, 0.71 (0.36, 1.39)	0.31
Pancreatic complications [9,34,35],[b]	3	1157	2324	RR, 1.67 (0.40, 6.90)	0.48
Chyle leakage [34,35]	2	1143	2296	RR, 0.34 (0.01, 8.31)	0.51
Overall surgical adverse events [5,7–9,34–36],[c]	7	1308	2626	RR, 0.69 (0.53, 0.91)	0.008*
Respiratory complications [7–9,34–36],[d]	6	1226	2462	RR, 0.40 (0.20, 0.79)	0.009*
Learning curve					
Surgeon experience [34–36],[e]	3	1174	2359	–	–

LADG, laparoscopic-assisted distal gastrectomy; ODG, open distal gastrectomy; RR, risk ratio; MD, mean difference; 95% CI, 95% confidence interval.

[a] Including: intraabdominal, intraluminal and anastomotic bleeding.

[b] Including: pancreatitis, pancreatic fistula and pancreatic injury.

[c] Including: wound infection, wound dehiscence, anastomotic stenosis, anastomotic leakage, postoperative bleeding, postoperative obstruction/ileus, delayed gastric emptying, intraabdominal abscess/fluid collection, pancreatic complications, and chyle leakage.

[d] Including: pneumonia, bronchiectasis, pulmonary atelectasis and so on.

[e] Trials which have mentioned the details of the least cases experience of the surgeons.

operative blood loss, postoperative pain, overall risk of surgical adverse events, and respiratory complications [5–9,34–36]. Those benefits may be attributed to the less invasive nature of LADG compared with ODG, which contributed to enhanced recovery after surgery. The reason for less operative blood loss in LADG patients may be attributed to the magnified view through the monitor, which permits meticulous dissection to prevent unexpected bleeding, thus preventing interference with surgical vision by blood accumulation. Intra-abdominal bleeding may also lead to reoperation [35]. LADG was also associated with a shorter time to first flatus, and a tendency toward a shorter duration of postoperative hospital stay, although the difference did not reach significance (MD −1.02, 95% CI: −2.06 to 0.01; $P = 0.05$) [5–9,34–36]. No procedure-associated differences in reoperation or operation-related deaths were found, which supported the safety of LADG. The current evidence supports the surgical safety and rapid recovery of LADG.

Oncological safety is of great importance for surgical treatment of

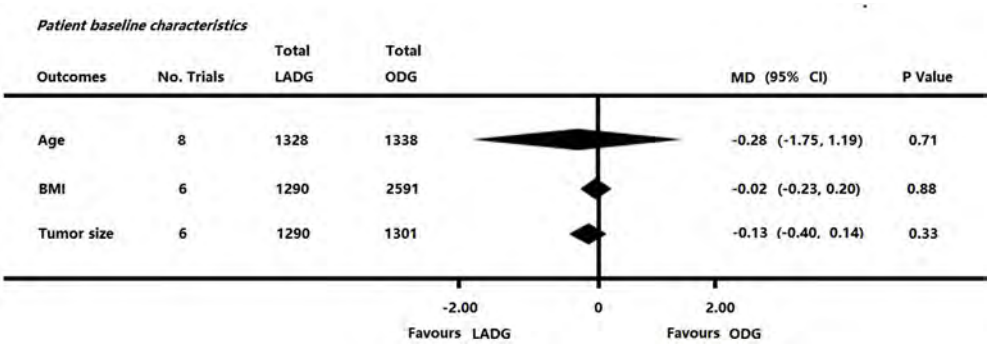

Fig. 3. Patient baseline characteristics.

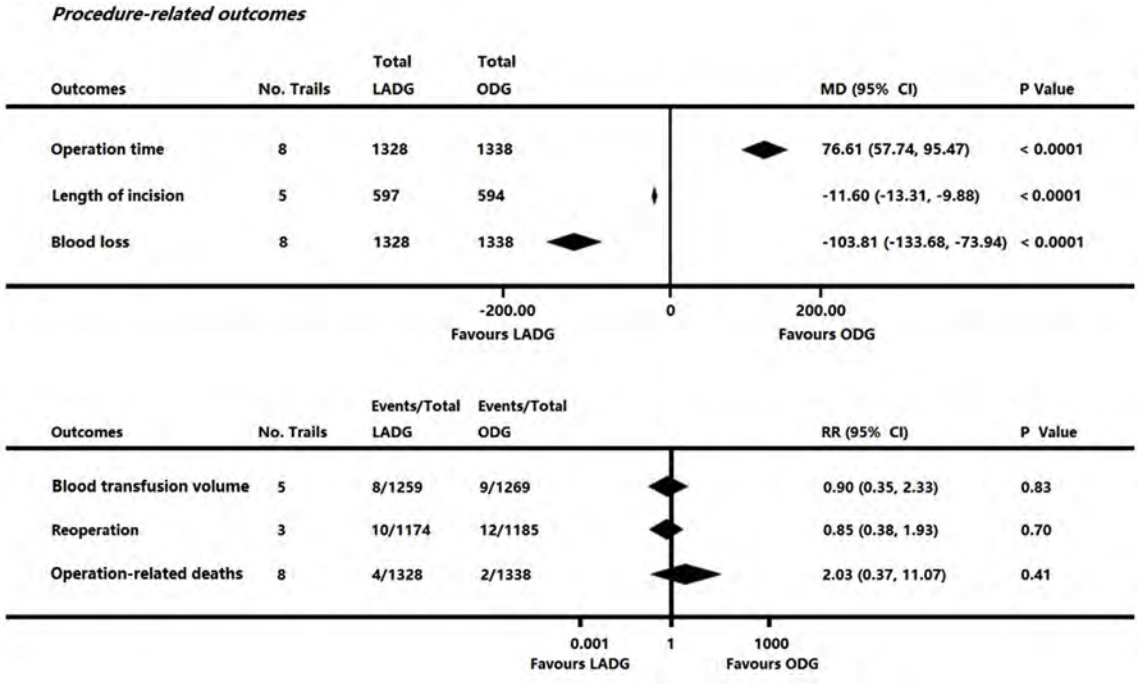

Fig. 4. Procedure-related outcomes.

gastric cancer. Lymph node metastasis is a frequent occurrence in gastric cancer, and adequate nodal harvest is the key step in distal gastrectomy. Lymph nodes dissection is also important for staging, as a larger total lymph node count may have a survival benefit [52]. Compared with ODG, LADG had a mean reduction of 2.2 harvested nodes. The included RCTs reported an average of at least 20.2 harvested nodes with LADG and 24.9 with ODG respectively, both of which are more than the minimum of 15 recommended by the current gastric cancer guidelines of the Union for International Cancer Control/American Joint Cancer Committee, the Japanese Gastric Cancer Association, and the European Society for Medical Oncology [5–9,34–36,53–59]. This meta-analysis found no procedure-related difference in risk of recurrence, or the number of patients with positive lymph nodes i.e., metastasis in one or more regional lymph nodes [5–9,34–36]. Similar results were reported in the included RCTs [5–9,34–36]. LADG and ODG thus had equivalent oncology safety, which supports LADG as an alternative to ODG for early gastric cancer. Importantly, although LADG had a mean reduction of 2.2 harvested nodes compared with ODG, the oncological outcomes were comparable. One plausible explanation might be that most patients included in current study were cT1 cases. Patients with cT1 gastric cancer are less likely to experience lymph node metastasis compared with those with T2 or more advanced tumors and might have better survival after gastrectomy.

This meta-analysis comprehensively and systematically screened the currently available evidence, which supports LADG as an alternative to ODG for early gastric cancer. However, caution still should be exercised in patients with node-positive early gastric cancer, as most patients included in this analysis were node negative; the percentage of node-positive cases in the selected studies ranged from 0% to 15.8%. Consequently, current evidence suggests that LADG has a high role to play in node-negative cases due to better short-term outcomes but less nodal harvest. LADG can be considered for node-positive cases by experienced surgeons in high-volume centers. The evidence warrants further trials in node-positive early gastric cancer cases, advanced gastric cancer cases, or cases in western countries.

Japan and South Korea have implemented gastric cancer screening programs [1,12–14,60–65]. Because gastric cancer is often diagnosed at an advanced stage, early detection may be the most effective intervention. Patients will benefit from a diagnosis at less advanced stage because of screening programs aiming at early detection. Screening by diagnostic endoscopy, histologic evaluation of biopsies, endoscopic ultrasonography [66], and accurate staging can be used to confirm the presence of abnormal or enlarged lymph nodes likely to harbor cancer. If no suspicion of node-positive cancer is found in an early stage patient who is also not suitable for EMR or ESD [67–69], LADG can be recommended as an alternative to ODG. For early gastric cancer patients

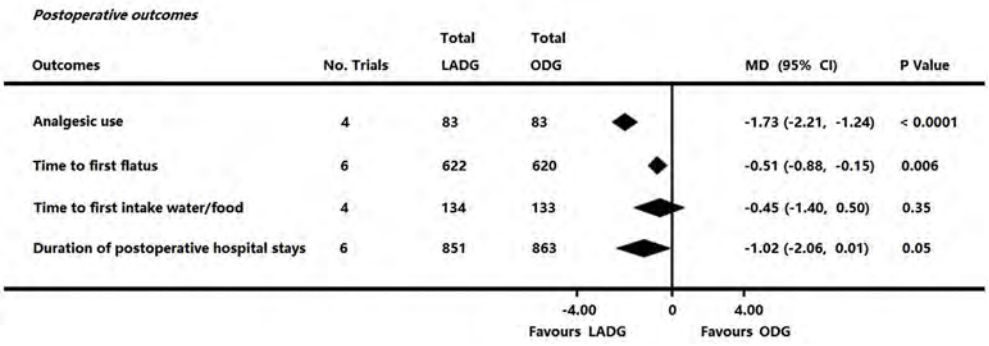

Fig. 5. Postoperative outcomes.

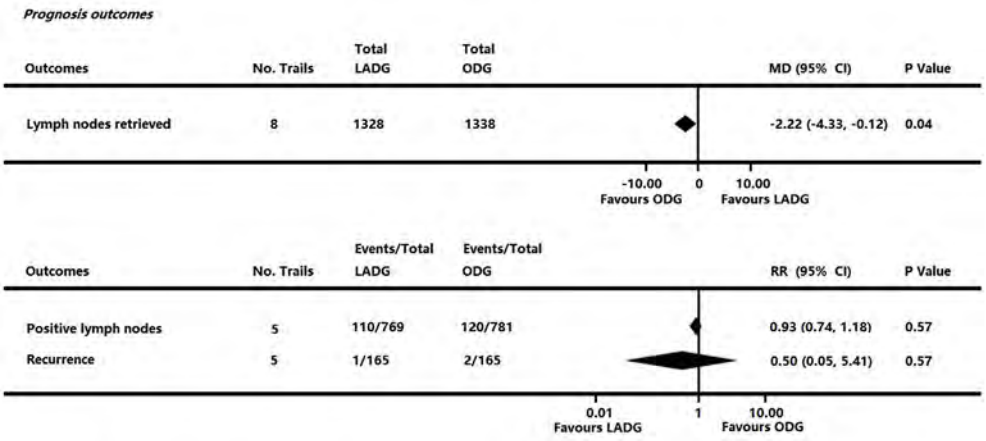

Fig. 6. Prognosis outcomes.

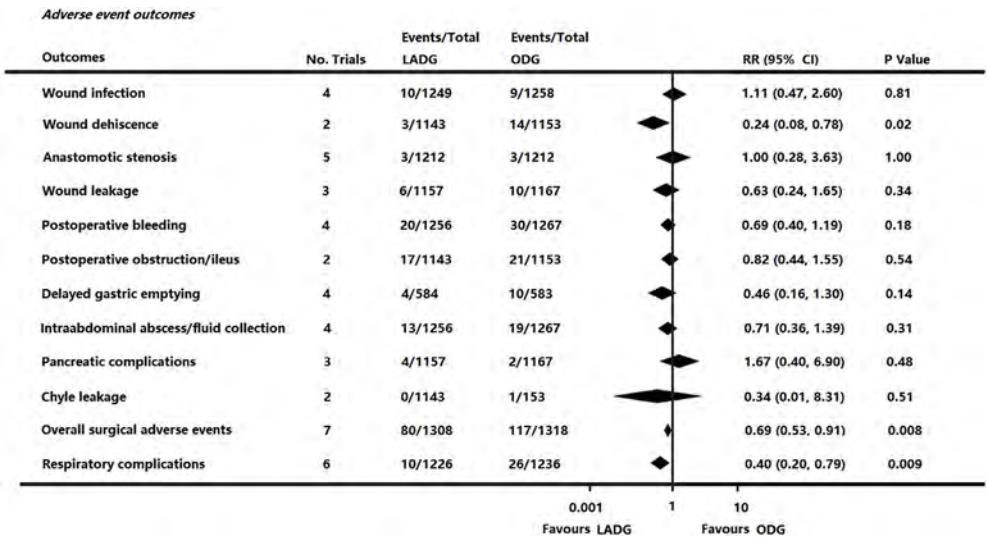

Fig. 7. Adverse event outcomes.

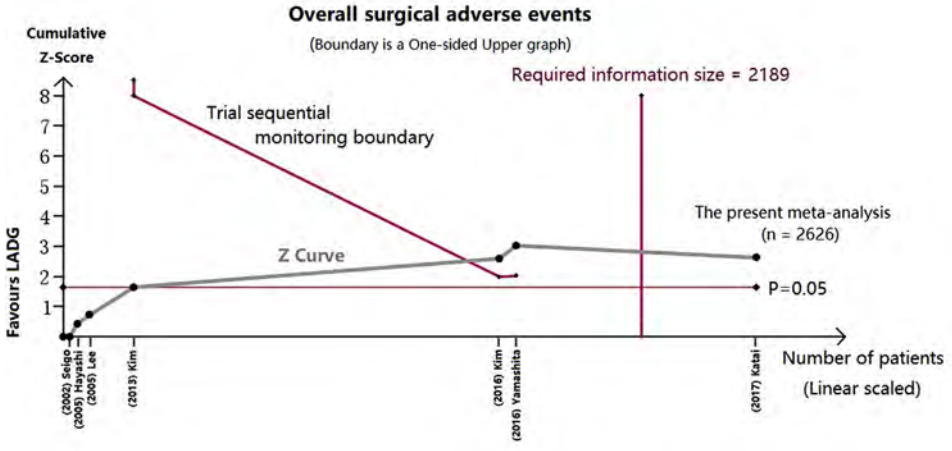

Fig. 8. Trial sequential analysis of overall surgical adverse events in seven RCTs comparing laparoscopic distal gastrectomy with open distal gastrectomy.

with suspected positive nodes, LADG is a choice which should be performed by experienced surgeons in high-volume centers.

Another important issue is how to define an experienced surgeon. The LADG learning curve requires significant training and expertise. Most RCTs included in the meta-analysis described surgeons as "experienced" or "well trained," but only three trials included the number of completed procedures when describing the experience of the surgeons [34–36]. Katai et al. reported "experience of at least 30 of both LADG and ODG operations" for LADG and "experience of at least 60 ODG operations" for ODG [34]. Kim et al. reported "experience of at least 50 each of LADG and ODG operations" for both LADG and ODG [35]. Yamashita et al. reported "experience of more than 100 LADG

Table 4
GRADE evidence profile.

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	LADG	ODG	Relative (95% CI)	Absolute		
Operation-related deaths [5–9,34–36]												
8 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	Serious [c]	None	4/1328 (0.3%)	2/1338 (0.1%) 0%	RR 2.03 (0.37–11.07)	2 more per 1000 (from 1 fewer to 15 more) –	⊕⊕OO LOW	CRITICAL
Lymph nodes retrieved [5–9,34–36] (Better indicated by lower values)												
8 RCTs	Randomised trials	Serious [b]	Serious [d]	No serious indirectness	No serious imprecision	None	1328	1338	–	MD 2.22 lower (4.33–0.12 lower)	⊕⊕OO LOW	CRITICAL
Recurrence [5,7–9,36]												
5 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	Serious [c]	Reporting bias [e]	1/165 (0.6%)	2/165 (1.2%) 0%	RR 0.50 (0.05–5.41)	6 fewer per 1000 (from 12 fewer to 53 more) –	⊕OOO VERY LOW	CRITICAL
Reoperation [34–36]												
3 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	Serious [c]	Reporting bias [e]	10/1174 (0.85%)	12/1185 (1%) 1.3%	RR 0.85 (0.38–1.93)	2 fewer per 1000 (from 6 fewer to 9 more) 2 fewer per 1000 (from 8 fewer to 12 more)	⊕OOO VERY LOW	CRITICAL
Overall surgical adverse events [5,7–9,34–36], [a]												
7 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	No serious imprecision	Reporting bias [e]	80/1308 (6.1%)	117/1318 (8.9%) 8.7%	RR 0.69 (0.53–0.91)	28 fewer per 1000 (from 8 fewer to 42 fewer) 27 fewer per 1000 (from 8 fewer to 41 fewer)	⊕⊕OO LOW	CRITICAL
Time to first flatus [5,7–9,34,36] (Better indicated by lower values)												
6 RCTs	Randomised trials	Serious [b]	Serious [f]	No serious indirectness	No serious imprecision	Reporting bias [e]	622	620	–	MD 0.51 lower (0.88–0.15 lower)	⊕OOO VERY LOW	IMPORTANT

GRADE, Grading of Recommendations Assessment, Development and Evaluation; RCTs, randomized controlled trials; LADG, laparoscopic-assisted distal gastrectomy; ODG, open distal gastrectomy; RR, risk ratio; MD, mean difference; 95% CI, 95% confidence interval; No, number.

GRADE Working Group grades of evidence: High quality: Further research is very unlikely to change our confidence in the estimate of effect; **Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; **Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; **Very low quality:** We are very uncertain about the estimate.

[a] Including: wound infection, wound dehiscence, anastomotic stenosis, anastomotic leakage, postoperative bleeding, postoperative obstruction/ileus, delayed gastric emptying, intraabdominal abscess/fluid collection, pancreatic complications, and chyle leakage.

[b] Only one RCT included in the present study applied single-blind methods (blind to patients).

[c] The 95% confidence interval (95% CI) for the total effect was too wide.

[d] Heterogeneity ($I^2 = 70\%$, $P = 0.002$) was found.

[e] High risk of publication bias.

[f] Heterogeneity ($I^2 = 92\%$, $P < 0.001$) was found.

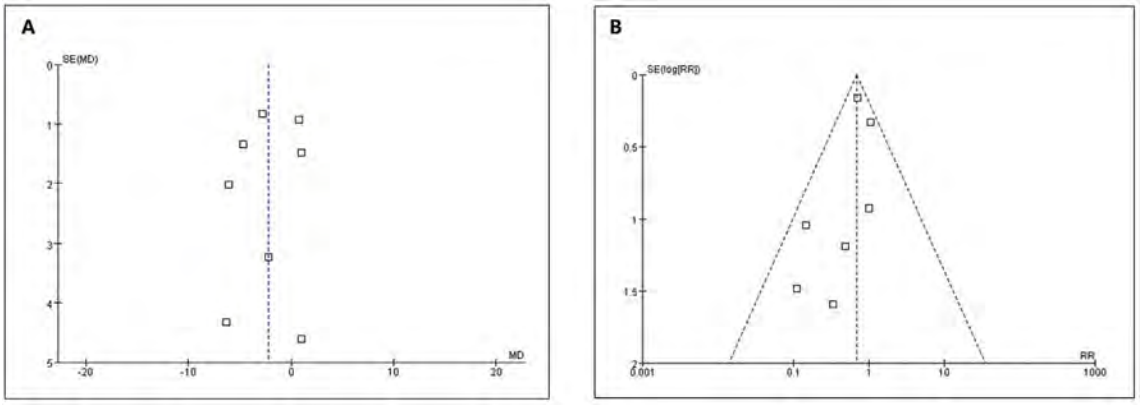

Fig. 9. A Funnel plot for publication bias in lymph nodes retrieved; **B** Funnel plot for publication bias in overall surgical adverse events.

operations" for LADG and "experience of more than 500 ODG operations" for ODG [36]. The fewest procedures performed by experienced surgeons ranged from 30 to 100 cases for LADG, and 50 to 500 cases for ODG [34–36]. Nevertheless, it is still difficult to define the minimum LADG or ODG procedures required as the criterion that defines an experienced LADG surgeon. The LADG learning curve may influence clinical outcomes because it is still relatively new and a technically complex procedure, especially for inexperienced surgeons. The current literature may indicate a minimum experience of no less than 30 LADG and 50 ODG procedures is required [34–36]. Future trials are still needed to confirm the "experienced" requirement.

The number of elderly patients with gastric cancer is increasing because life expectancy is consistently increasing. Compared with younger patients, elderly patients may have an increased surgical risk because of poorer nutritional and functional status, which may result in higher postoperative morbidity and mortality. Importantly, laparoscopy-assisted procedures are associated with less trauma, faster recovery, and similar surgical and oncological safety, compared with open procedures. Therefore, interest in the use of LADG in elderly patients with gastric cancer is increasing. In a meta-analysis of non-RCTs, Zong et al. reported that LADG significantly reduced both operation-related and systemic morbidities and did not increase cardiopulmonary or mental dysfunction compared with ODG in elderly gastric cancer patients [70]. Another meta-analysis of observational studies by Wang et al. demonstrated that compared with ODG, LADG was a feasible and safe approach for elderly patients with gastric cancer. It was associated with less blood loss, faster postoperative recovery, and reduced postoperative morbidity [71]. In the latest meta-analysis of observational studies, Pan et al. reported that the outcomes of LADG in elderly patients were comparable to those in younger patients and that age alone should not preclude LADG in elderly patients with gastric cancer [72].

The strengths of this meta-analysis including adequate power with 2666 participants. All included trials were high quality RCTs. Other strengths were following the PRISMA and GRADE evidence profiles, both of which were recommended by the Cochrane Collaboration [33]. Furthermore, TSA was performed to reduce the influence of random error and confirm whether the evidence was reliable and conclusive. Limitations might exist in this study. Firstly, it included a small percentage of node-positive cancers among the largely node-negative treated population that could have influenced the outcomes. Secondly, all included trials were from Japan and South Korea, none were from China, which has one of the highest incidences of gastric cancer worldwide. Thirdly, bias may have been introduced by differences in the LADG experience and learning status of the surgeons who performed the procedures. Fourthly, none of the trials reported quality of life scores or economic assessments, which are areas of concern. Fifth, data of long-term overall survival (OS) and disease-free survival (DFS) outcomes are not available in the current literature which are important. Finally, the quality of the evidence of the included trials was relatively low, as assessed by the GRADE evidence profile.

In conclusion, the currently available evidence supports LADG an alternative to ODG for Asian patients with early gastric cancer because of similar mortality and oncological safety, better surgical safety, decreased operative morbidity, less trauma, and accelerated recovery. It has a high role to play in node-negative cases due to better short-term outcomes but less nodal harvest. It should be performed by experienced surgeons in high-volume centers, and caution should be exercised with node-positive cases and cases in western countries.

Ethical Approval

There is no need to gain Ethical Approval for this meta-analysis.

Sources of funding

This study was supported in part by Japan China Sasakawa Medical Fellowship.

Author contribution

Zhang C.D., Yamashita H., Zhang S., and Seto Y. are the authors responsible for the study's conception and design. Zhang C.D. and Zhang S., are responsible for acquisition of data, and analysis and interpretation of data. Seto Y. contributed most importantly by giving the final approval.

Conflict-of-interest statement

The authors have declared that there are no conflicts of interest.

Registration unique identifying number

UIN is reviewregistry518.

Guarantor

Yasuyuki Seto and Chun-Dong Zhang.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijsu.2018.05.733.

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, et al., Cancer incidence

and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Canc. 136 (2015) E359–E386.
[2] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, et al., Cancer statistics in China, 2015, CA Cancer J. Clin 66 (2016) 115–132.
[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA Cancer J. Clin 68 (2018) 7–30.
[4] S. Kitano, Y. Iso, M. Moriyama, K. Sugimachi, Laparoscopy-assisted billroth I gastrectomy, Surg. Laparosc. Endosc. 4 (1994) 146–148.
[5] Y.W. Kim, H.M. Yoon, Y.H. Yun, B.H. Nam, B.W. Eom, et al., Long-term outcomes of laparoscopy-assisted distal gastrectomy for early gastric cancer: result of a randomized controlled trial (COACT 0301), Surg. Endosc. 27 (2013) 4267–4276.
[6] S. Takiguchi, Y. Fujiwara, M. Yamasaki, H. Miyata, K. Nakajima, et al., Laparoscopy-assisted distal gastrectomy versus open distal gastrectomy. A prospective randomized single-blind study, World J. Surg. 37 (2013) 2379–2386.
[7] H. Hayashi, T. Ochiai, H. Shimada, Y. Gunji, Prospective randomized study of open versus laparoscopy-assisted distal gastrectomy with extraperigastric lymph node dissection for early gastric cancer, Surg. Endosc. 19 (2005) 1172–1176.
[8] J.H. Lee, H.S. Han, J.H. Lee, A prospective randomized study comparing open vs laparoscopy-assisted distal gastrectomy in early gastric cancer: early results, Surg. Endosc. 19 (2005) 168–173.
[9] S. Kitano, N. Shiraishi, K. Fujii, K. Yasuda, M. Inomata, et al., A randomized controlled trial comparing open vs laparoscopy-assisted distal gastrectomy for the treatment of early gastric cancer: an interim report, Surgery (St Louis) 131 (2002) S306–S311.
[10] K. Nakamura, H. Katai, J. Mizusawa, T. Yoshikawa, M. Ando, et al., A phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastric Cancer (JCOG0912), Jpn, J. Clin. Oncol. 43 (2013) 324–327.
[11] S. Sakuramoto, K. Yamashita, S. Kikuchi, N. Futawatari, N. Katada, et al., Laparoscopy versus open distal gastrectomy by expert surgeons for early gastric cancer in Japanese patients: short-term clinical outcomes of a randomized clinical trial, Surg. Endosc. 27 (2013) 1695–1705.
[12] Y.W. Kim, Y.H. Baik, Y.H. Yun, B.H. Nam, D.H. Kim, et al., Improved quality of life outcomes after laparoscopy-assisted distal gastrectomy for early gastric cancer: results of a prospective randomized clinical trial, Ann. Surg. 248 (2008) 721–727.
[13] H.H. Kim, W.J. Hyung, G.S. Cho, M.C. Kim, S.U. Han, et al., Morbidity and mortality of laparoscopic gastrectomy versus open gastrectomy for gastric cancer: an interim report–a phase III multicenter, prospective, randomized Trial (KLASS Trial), Ann. Surg. 251 (2010) 417–420.
[14] K. Fujii, K. Sonoda, K. Izumi, N. Shiraishi, Y. Adachi, et al., T lymphocyte subsets and Th1/Th2 balance after laparoscopy-assisted distal gastrectomy, Surg. Endosc. 17 (2003) 1440–1444.
[15] L. Zong, M. Abe, Y. Seto, J. Ji, The challenge of screening for early gastric cancer in China, Lancet 388 (2016) 2606.
[16] K. Sumiyama K, Past and current trends in endoscopic diagnosis for early stage gastric cancer in Japan, Gastric Cancer 20 (2017) 20–27.
[17] C. Hamashima, Cancer screening guidelines and policy making: 15 years of experience in cancer screening guideline development in Japan, Jpn. J. Clin. Oncol. 48 (2018) 278–286.
[18] K. Misawa, M. Fujiwara, M. Ando, S. Ito, Y. Mochizuki, et al., Long-term quality of life after laparoscopic distal gastrectomy for early gastric cancer: results of a prospective multi-institutional comparative trial, Gastric Cancer 18 (2015) 417–425.
[19] J.H. Lee, B.H. Nam, K.W. Ryu, S.Y. Ryu, Y.K. Park, et al., Comparison of outcomes after laparoscopy-assisted and open total gastrectomy for early gastric cancer, Br. J. Surg. 102 (2015) 1500–1505.
[20] T. Yoshikawa, H. Cho, Y. Rino, Y. Yamamoto, M. Kimura, et al., A prospective feasibility and safety study of laparoscopy-assisted distal gastrectomy for clinical stage I gastric cancer initiated by surgeons with much experience of open gastrectomy and laparoscopic surgery, Gastric Cancer 16 (2013) 126–132.
[21] H. Katai, M. Sasako, H. Fukuda, K. Nakamura, N. Hiki, JCOG Gastric Cancer Surgical Study Group, Safety and feasibility of laparoscopy-assisted distal gastrectomy with suprapancreatic nodal dissection for clinical stage I gastric cancer: a multicenter phase II trial (JCOG 0703), Gastric Cancer 13 (2010) 238–244.
[22] Y. Kurokawa, H. Katai, H. Fukuda, M. Sasako, Gastric cancer surgical study group of the Japan clinical oncology group, phase II study of laparoscopy-assisted distal gastrectomy with nodal dissection for clinical stage I gastric cancer: Japan clinical oncology group study JCOG0703, Jpn. J. Clin. Oncol. 38 (2008) 501–503.
[23] J.Y. An, G.U. Heo, J.H. Cheong, W.J. Hyung, S.H. Choi, et al., Assessment of open versus laparoscopy-assisted gastrectomy in lymph node-positive early gastric cancer: a retrospective cohort analysis, J. Surg. Oncol. 102 (2010) 77–81.
[24] X.Z. Chen, J.K. Hu, K. Yang, L. Wang, Q.C. Lu, Short-term evaluation of laparoscopy-assisted distal gastrectomy for predictive early gastric cancer: a meta-analysis of randomized controlled trials, Surg. Laparosc. Endosc. Percutaneous Tech. 19 (2009) 277–284.
[25] J.S. Peng, H. Song, Z.L. Yang, J. Xiang, D.C. Diao, et al., Meta-analysis of laparoscopy-assisted distal gastrectomy and conventional open distal gastrectomy for early gastric cancer, Chin. J. Canc. 29 (2010) 349–354.
[26] H. Ohtani, Y. Tamamori, K. Noguchi, T. Azuma, S. Fujimoto, et al., A meta-analysis of randomized controlled trials that compared laparoscopy-assisted and open distal gastrectomy for early gastric cancer, J. Gastrointest. Surg. 14 (2010) 958–964.
[27] Y. Liang, G. Li, P. Chen, J. Yu, C. Zhang, Laparoscopic versus open gastrectomy for early distal gastric cancer: a meta-analysis, ANZ, J. Surg. 81 (2011) 673–680.
[28] Y.K. Zeng, Z.L. Yang, J.S. Peng, H.S. Lin, L. Cai, Laparoscopy-assisted versus open distal gastrectomy for early gastric cancer: evidence from randomized and non-randomized clinical trials, Ann. Surg. 256 (2012) 39–52.
[29] C.D. Zhang, S.C. Chen, Z.F. Feng, Z.M. Zhao, J.N. Wang, et al., Laparoscopic versus open gastrectomy for early gastric cancer in Asia: a meta-analysis, Surg. Laparosc. Endosc. Percutaneous Tech. 23 (2013) 365–377.
[30] Y. Wang, S. Wang, Z.Q. Huang, W.P. Chou, Meta-analysis of laparoscopy assisted distal gastrectomy and conventional open distal gastrectomy for EGC, Surgeon 12 (2014) 53–58.
[31] Y. Deng, Y. Zhang, T.K. Guo TK, Laparoscopy-assisted versus open distal gastrectomy for early gastric cancer: a meta-analysis based on seven randomized controlled trials, Surg. Oncol 24 (2015) 71–77.
[32] W. Lu, J. Gao, J. Yang, Y. Zhang, W. Lv, et al., Long-term clinical outcomes of laparoscopy-assisted distal gastrectomy versus open distal gastrectomy for early gastric cancer: a comprehensive systematic review and meta-analysis of randomized control trials, Medicine 95 (2016) e3986.
[33] J.P.T. Higgins, S. Green, Cochrane Handbook for systematic Reviews of interventions version 5.1.0. The Cochrane collaboration, Available at: http://handbook.cochrane.org (2011).
[34] H. Katai, J. Mizusawa, H. Katayama, M. Takagi, T. Yoshikawa, et al., Short-term surgical outcomes from a phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastriccancer: Japan Clinical Oncology Group Study JCOG0912, Gastric Cancer 20 (2017) 699–708.
[35] W. Kim, H.H. Kim, S.U. Han, M.C. Kim, W.J. Hyung, Korean laparo-endoscopic gastrointestinal surgery study (KLASS) group, decreased morbidity of laparoscopic distal gastrectomy compared with open distal gastrectomy for stage I gastric cancer: short-term outcomes from a multicenter randomized controlled trial (KLASS-01), Ann. Surg. 263 (2016) 28–35.
[36] K. Yamashita, S. Sakuramoto, S. Kikuchi, N. Futawatari, N. Katada, et al., Laparoscopic versus open distal gastrectomy for early gastric cancer in Japan: long-term clinical outcomes of a randomized clinical trial, Surg. Today 46 (2016) 741–749.
[37] J.P. Higgins, S.G. Thompson, J.J. Deeks, D.G. Altman, Measuring inconsistency in meta-analyses, BMJ 327 (2003) 557–560.
[38] G.H. Guyatt, A.D. Oxman, G.E. Vist, R. Kunz, Y. Falck-Ytter, GRADE Working Group, GRADE: an emerging consensus on rating quality of evidence and strength of recommendations, BMJ 336 (2008) 924–926.
[39] K. Thorlund, J. Engstrøm, J. Wetterslev, J. Brok, G. Imberger, et al., User Manual for Trial Sequential Analysis (TSA), Denmark: Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen, 2011 1–115.
[40] J. Wetterslev, K. Thorlund, J. Brok, C. Gluud, Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis, Journal. of. Clinical. Epidemiology 61 (2008) 64–75.
[41] J. Brok, K. Thorlund, C. Gluud, J. Wetterslev, Trial sequential analysis reveals insufficient information size and potentially false positive results in many meta-analyses, Journal. of. Clinical. Epidemiology 61 (2008) 763–769.
[42] K. Thorlund, P.J. Devereaux, J. Wetterslev, G. Guyatt, J.P. Ioannidis, et al., Can trial sequential monitoring boundaries reduce spurious inferences from meta-analyses? International. Journal. of. Epidemiology 38 (2009) 276–286.
[43] J. Brok, K. Thorlund, J. Wetterslev, C. Gluud, Apparently conclusive meta-analyses may be inconclusive - trial sequential analysis adjustment of random error risk due to repetitive testing of accumulating data in apparently conclusive neonatal meta-analyses, International. Journal. of. Epidemiology 38 (2009) 287–298.
[44] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman DG, P.R.I.S.M.A. Group, Preferred reporting Items for systematic Reviews and meta-analyses: the PRISMA statement, BMJ 339 (2009) b2535.
[45] J.P. Higgins, D.G. Altman, P.C. Gøtzsche, P. Jüni, D. Moher, Cochrane bias methods group; Cochrane statistical methods group, the Cochrane Collaboration's tool for assessing risk of bias in randomised trials, BMJ 343 (2011) d5928.
[46] J.A. Sterne, A.J. Sutton, J.P. Ioannidis, N. Terrin, D.R. Jones, et al., Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials, BMJ 343 (2011) d4002.
[47] D. Luo, X. Wan, J. Liu, T. Tong, Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range, Stat. Meth. Med. Res. 27 (2018) 1785–1805.
[48] X. Wan, W. Wang, J. Liu, T. Tong, Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range, BMC, Med. Res. Methodol 14 (2014) 135.
[49] C.G. Huscher, A. Mingoli, G. Sgarzini, A. Sansonetti, M. Di Paola, et al., Laparoscopic versus open subtotal gastrectomy for distal gastric cancer: five-year results of a randomized prospective trial, Ann. Surg. 241 (2005) 232–237.
[50] H.J. Lee, H.H. Kim, M.C. Kim, S.Y. Ryu, W. Kim, Korean Laparoscopic Gastrointestinal Surgery Study Group, the impact of a high body mass index on laparoscopy assisted gastrectomy for gastric cancer, Surg. Endosc. 23 (2009) 2473–2479.
[51] J.H. Jung, S.Y. Ryu, M.R. Jung, Y.K. Park, O. Jeong, Laparoscopic distal gastrectomy for gastric cancer in morbidly obese patients in South Korea, J. Gastric. Cancer 14 (2014) 187–195.
[52] D.D. Smith, R.R. Schwarz, R.E. Schwarz, Impact of total lymph node count on staging and survival after gastrectomy for gastric cancer: data from a large US-population database, J. Clin. Oncol. 23 (2005) 7114–7124.
[53] Japanese Gastric Cancer Association, Japanese gastric cancer treatment guidelines 2014 (ver. 4), Gastric Cancer 20 (2017) 1–19.
[54] J.A. Ajani, D.J. Bentrem, S. Besh, T.A. D'Amico, P. Das, National Comprehensive Cancer Network, Gastric cancer, version 2.2013: featured updates to the NCCN Guidelines, J. Natl. Compr. Canc. Netw. 11 (2013) 531–546 2013.
[55] J.A. Ajani, T.A. D'Amico, K. Almhanna, D.J. Bentrem, J. Chao, et al., Gastric cancer, version 3. 2016, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Canc. Netw. 14 (2016) 1286–1312.
[56] E.C. Smyth, M. Verheij, W. Allum, D. Cunningham, A. Cervantes, ESMO Guidelines,

Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 27 (2016) v38–v49.
[57] T. Waddell, M. Verheij, W. Allum, D. Cunningham, A. Cervantes, et al., European society for medical oncology (ESMO); european society of surgical oncology (ESSO); european society of radiotherapy and oncology (ESTRO), gastric cancer: ESMO-ESSO-ESTRO clinical practice guidelines for diagnosis, treatment and follow-up, Eur. J. Surg. Oncol. 40 (2014) 584–591.
[58] I. Songun, H. Putter, E.M. Kranenbarg, M. Sasako, C.J. van de Velde, Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial, Lancet Oncol. 11 (2010) 439–449.
[59] R.E. Schwarz, D.D. Smith, Clinical impact of lymphadenectomy extent in resetable gastric cancer of advanced stage, Ann. Surg Oncol. 14 (2007) 317–328.
[60] O. Dohi, N. Yagi, A. Majima, Y. Horii, T. Kitaichi, Diagnostic ability of magnifying endoscopy with blue laser imaging for early gastric cancer: a prospective study, Gastric Cancer 20 (2017) 297–303.
[61] Q. Zhang, F. Wang, Z.Y. Chen, Z. Wang, F.C. Zhi, et al., Comparison of the diagnostic efficacy of white light endoscopy and magnifying endoscopy with narrow band imaging for early gastric cancer: a meta-analysis, Gastric Cancer. 19(206) 543–552.
[62] T. Nagahama, K. Yao, N. Uedo, H. Doyama, T. Ueo, et al., Delineation of the extent of early gastric cancer by magnifying narrow-band imaging and chromoendoscopy: a multicenter randomized controlled trial, Endoscopy 50 (2018) 566–576.
[63] K. Sumiyama, Past and current trends in endoscopic diagnosis for early stage gastric cancer in Japan, Gastric Cancer 20 (2017) 20–27.
[64] S. Yamada, H. Doyama, K. Yao, N. Uedo, Y. Ezoe, et al., An efficient diagnostic strategy for small, depressed early gastric cancer with magnifying narrow-band imaging: a post-hoc analysis of a prospective randomized controlled trial, Gastrointest. Endosc. 79 (2014) 55–63.
[65] Y. Ezoe, M. Muto, N. Uedo, H. Doyama, K. Yao, et al., Magnifying narrowband imaging is more accurate than conventional white-light imaging in diagnosis of gastric mucosal cancer, Gastroenterology 141 (2011) 2017–2025.
[66] R.M. Kwee, T.C. Kwee, Imaging in local staging of gastric cancer: a systematic review, J. Clin. Oncol. 25 (2007) 2107–2116.
[67] R. Soetikno, T. Kaltenbach, R. Yeh, T. Gotoda, Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract, J. Clin. Oncol. 23 (2005) 4490–4498.
[68] S. Nakamoto, Y. Sakai, J. Kasanuki, F. Kondo, Y. Ooka, et al., Indications for the use of endoscopic mucosal resection for early gastric cancer in Japan: a comparative study with endoscopic submucosal dissection, Endoscopy 41 (2009) 746–750.
[69] Y.M. Park, E. Cho, H.Y. Kang, J.M. Kim, The effectiveness and safety of endoscopic submucosal dissection compared with endoscopic mucosal resection for early gastric cancer: a systematic review and meta-analysis, Surg. Endosc. 25 (2011) 2666–2677.
[70] L. Zong, A. Wu, W. Wang, J. Deng, S. Aikou, et al., Feasibility of laparoscopic gastrectomy for elderly gastric cancer patients: meta-analysis of non-randomized controlled studies, Oncotarget 8 (2017) 51878–51887.
[71] J.F. Wang, S.Z. Zhang, N.Y. Zhang, Z.Y. Wu, J.Y. Feng, et al., Laparoscopic gastrectomy versus open gastrectomy for elderly patients with gastric cancer: a systematic review and meta-analysis, World J. Surg. Oncol. 14 (2016) 90.
[72] Y. Pan, K. Chen, W.H. Yu, H. Maher, S.H. Wang, et al., Laparoscopic gastrectomy for elderly patients with gastric cancer: a systematic review with meta-analysis, Medicine 97 (2018) e0007.

frontiers in Oncology

ORIGINAL RESEARCH
published: 15 January 2019
doi: 10.3389/fonc.2018.00671

Comparison of Different Lymph Node Staging Systems in Patients With Resectable Colorectal Cancer

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Background and Objectives: Currently, the United States Joint Commission on Cancer (AJCC) N staging, lymph node positive rate (LNR), and log odds of positive lymph nodes (LODDS) are the main lymph node (LN) staging systems. However, the type of LN staging system that is more accurate in terms of prognostic performance remains controversial. We compared the prognostic accuracy of the three staging systems in patients with CRC and determine the best choice for clinical applications.

Methods: From the Surveillance, Epidemiology, and End Results (SEER) database, 56,747 patients were identified who were diagnosed with CRC between 2004 and 2013. Akaike's Information Criterion (AIC) and Harrell's Consistency Index (c-index) were used to assess the relative discriminative abilities of different LN staging systems.

Results: In 56,747 patients, when using classification cut-off values for evaluation, the LNR of Rosenberg et al. showed significantly better predictive power, especially when the number of dissected lymph nodes (NDLN) were insufficient. When analyzed as a continuous variable, the LODDS staging system performed the best and was not affected by the NDLN.

Conclusions: We suggest that the LNR of Rosenberg et al. should be introduced into the AJCC system as a supplement when the NDLN is insufficient until the optimal LODDS cut-off values are calculated.

Keywords: log odds, lymph node ratio, N staging, colorectal cancer, survival analysis

OPEN ACCESS

Edited by:
Robert Rosenberg, Kantonsspital Baselland, Switzerland

Reviewed by:
Mirko Omejc, University of Ljubljana, Slovenia
Vladimir Cuk, Clinical Hospital Center Zvezdara, Serbia

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Specialty section:
This article was submitted to Gastrointestinal Cancers, a section of the journal Frontiers in Oncology

Received: *25 October 2018*
Accepted: *20 December 2018*
Published: *15 January 2019*

Citation:
Pei J-P, Zhang C-D, Fan Y-C and Dai D-Q (2019) Comparison of Different Lymph Node Staging Systems in Patients With Resectable Colorectal Cancer. Front. Oncol. 8:671. doi: 10.3389/fonc.2018.00671

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and women in the United States (1). Lymph node (LN) metastasis is an important prognostic factor associated with overall survival (OS) (2). Therefore, in order to accurately describe LN status, a variety of LN staging systems have been proposed. The most representative of these LN staging systems are the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) eighth edition N staging (3), lymph node ratio (LNR) and the log odds of positive lymph nodes (LODDS).

The goal of cancer staging systems is to group patients with similar prognosis. Rice et al. defined the characteristics of a good staging system as: (a) the patient survival rate decreases as the stage group increases (Monotonicity), (b) the groups have clearly different survival rates

(Distinctiveness), and (c) within a group, the survival rate is similar (Homogeneity) (4). Currently, the most widely accepted LN staging system is the AJCC/UICC 8th N staging, which is based on the absolute number of positive lymph nodes (NPLN). Its classification system is: pN0: no LN metastasis; pN1a: 1 metastatic LN; pN1b: 2–3 metastatic LNs; pN2a: 4–6 metastatic LNs; pN2b: ≥7 metastatic LNs (3).

Many studies have shown that OS is closely related to the NDLN in resectable surgery in patients with CRC, and a greater NDLN could provide more accurate staging and longer survival (5–7). The AJCC/UICC 8th N staging system recommends that at least 12 LNs in tumor specimens must be resectable and histopathologically evaluated to fully assess LN status. However, despite the availability of accurate recommendations, the recommended cut-off values for the NDLN needed varies widely among published studies, with the median ranging between 6 and 13, which results in staging migration and can affect further treatment for CRC (8, 9). In addition to surgeons, pathologists have also played a significant role in determining the status of LN in resected specimens (10). Therefore, in order to reduce staging migration, two new LN staging systems have been proposed.

LNR is defined as the ratio of NPLN relative to the NDLN. Recently, some scholars have reported that LNR has been shown to have a strong independent prognostic value in rectal and colon cancer (11, 12). These results were also shown in patients with lung, breast, and gastric cancer (13–15). Berger et al. first proposed that LNR has a higher prognostic impact in patients with colon cancer. They believed that LNR could reduce staging migration in patients with an insufficient NDLN (16). Rosenberg et al. also suggested that LNR should include routine histopathology reports because of their higher prognostic impact on colon cancer than AJCC/UICC N staging (17). However, some experts believe that when the NDLN is not sufficient, LNR cannot completely eliminate staging migration (18, 19). In addition, when LNR is an extreme value (LNR = 0 or 1), it does not accurately predict prognosis (12).

LODDS is another innovative N staging system. LODDS is defined as the logarithm of the ratio between the probability of being a positive LN and the probability of being a negative LN when an LN is retrieved (5, 20, 21). The formula for the LODDS system is log{(NPLN + 0.5)/(NDLN - NPLN + 0.5)}. "0.5" appears twice in the formula to avoid dividing by 0 and avoid having many patients with a LODDS of 0. According to previous reports, the use of LODDS has reduced the risk of staging migration in gastric, breast, colon, and pancreatic cancer in recent years (22–25). After comparing the prognostic utility of the LODDS system with the LNR system and AJCC/UICC N staging in patients with CRC, Persiani et al. showed that the LODDS system performed better (24). Wang et al. used the Surveillance, Epidemiology, and End Results (SEER) data to study the LODDS system in stage III colon cancer cases and concluded that LODDS also performed better than LNR and AJCC/UICC N staging in predicting prognosis (26).

The aim of this study was to compare the ability of different LN staging systems to predict OS in patients with resectable CRC to identify the most accurate system for application in clinical practice.

MATERIALS AND METHODS

Patients

In this retrospective analysis, we used data from the SEER linked database. The SEER Program of the National Cancer Institute is an authoritative source of information on cancer incidence and survival in the United States (U.S.) that is updated annually. SEER currently collects and publishes cancer incidence and survival data from population-based cancer registries covering approximately 34.6 percent of the U.S. population. Data from SEER was used to identify patients with CRC diagnosed between 2004 and 2013. Among the 90,529 patients diagnosed with CRC between these years, patients with the following characteristics were included: (a) the patients were over 18 years old; (b) CRC was the first and only malignant tumor; (c) surgical resection was performed; (d) there was complete staging information; and (e) no neoadjuvant chemoradiation was used in treatment. The final study sample contained 56,747 patients.

LN Staging Systems

We analyzed LNR and LODDS as both continuous and categorical variables. When used as categorical variables, different researchers have developed different optimal cut-off values. For the LNR staging system, we used cut-off values from Berger et al. and Rosenberg et al. Berger et al. considered 0.05, 0.19, and 0.39 as the best cut-off values, and divided the LNR into four groups as follows: LNR1 < 0.05; $0.05 \leq$ LNR2 < 0.19; $0.19 \leq$ LNR3 < 0.39; and $0.39 \leq$ LNR4 ≤ 1.00 (16). Rosenberg et al. calculated the best cut-off values between groups as 0.17, 0.41 and 0.69, and divided the LNR into five subgroups as follows: LNR0 $= 0.00$; $0.01 \leq$ LNR1 ≤ 0.17; $0.18 \leq$ LNR2 ≤ 0.41; $0.42 \leq$ LNR3 ≤ 0.69; and LNR4 ≥ 0.70 (17). For the LODDS staging system, we used the ideal cut-off values from Persiani et al. and Wang et al. Persiani et al. divided LODDS into three groups as follows: LODDS1 ≤ -1.36; $-1.36 <$ LODDS2 ≤ -0.53; LODDS3 > -0.53 (24). Wang et al. divided LODDS into five groups as follows: LODDS1 < -2.2; $-2.2 \leq$ LODDS2 < -1.1; $-1.1 \leq$ LODDS3 < 0.0; $0.0 \leq$ LODDS4 < 1.1; LODDS5 ≥ 1.1 (26) (**Table 1**).

Statistical Analysis

We used the Kaplan-Meier method to estimate OS and tested it using the log-rank procedure. Odds ratio (OR) and 95% confidence intervals (95% CI) are presented. We used the Akaike Information Criterion (AIC) and the Harrell Consistency Index (c-index) to assess the relative discriminative power of different LN staging systems. A value of $c = 0.5$ indicates no predictive power, and a value of $c = 1$ indicates complete differentiation. In general, a predictive model with a low AIC indicates a better model fit, while a high c-index indicates a better discriminating ability. All analyses were carried out with SPSS version 22.0 and R version 3.50. For all analysis, $P < 0.05$ was considered significant, and all tests were two-tailed.

RESULTS

Patient Characteristics

Table 2 shows clinical and histopathological characteristics for the study population. The cohort consisted of 27,507 males (48.5%) and 29,240 females (51.5%). The median age ± standard deviation was 66.0 ± 13.3 years. There were 22,723 (40.5%) patients with CRC who had LN metastases and 34,024 (59.5%) patients with no LN metastases. The mean ± standard deviation of NDLN and NPLN in the whole cohort were 16.9 ± 9.8 and 1.6 ± 3.3, respectively. 10,613 (18.7%) subjects had tumor located in the rectum and 46,134 (81.3%) were in the colon. In the univariate analysis, the age of diagnosis, histological grade, pT stage, tumor size, and NDLN were significantly correlated with prognosis.

Survival

Survival analysis was performed on the factors in the univariate analysis (**Figures 1A–G**). The 5-year OS of patients with an adequate NDLN was 79.7% and with an inadequate NDLN was 76.2% ($P < 0.001$; **Figure 1E**). The 5-year OS of patients with tumor located in the rectum was 78.3% and in the colon was 78.5%. The tumor location was not significant in predicting prognosis ($P = 0.763$; **Figure 1G**). Therefore, we grouped rectal and colon cancer together. The 5-year OS of different histological grades were 87.6% for well differentiated, 80.2% for moderately differentiated, 66.9% for poorly differentiated, and 65.4% for undifferentiated ($P < 0.001$; **Figure 1B**). No significant difference was found between poorly differentiated and undifferentiated tumors ($P = 0.148$). Kaplan-Meier survival curves and survival data based on different LN staging systems are shown in **Figure 2** and **Table 3** for all patients. The AJCC/UICC N staging system divided patients into five different prognostic groups and the 5-year OS for each subgroup were: pN0 = 87.2%, pN1a = 75.2%, pN1b = 68.1%, pN2a = 58.3%, and pN2b = 44.1% ($P < 0.001$; **Figure 2A**). The 5-year OS of the LNR subgroups according to the Rosenberg et al. criteria were LNR0 = 87.2%, LNR1 = 74.1%, LNR2 = 61.3%, LNR3 = 48.9%, and LNR4 = 33.0% ($P < 0.001$; **Figure 2B**), and the 5-year OS according to the Berger et al. criteria were LNR1 = 86.9%, LNR2 = 72.4%, LNR3 = 61.3%, and LNR4 = 44.3% ($P < 0.001$; **Figure 2C**). Finally, the 5-year OS of LODDS based on the classification by Wang et al. were LODDS1 = 91%, LODDS2 = 86.5%, LODDS3 = 69.7%, LODDS4 = 48.8%, and LODDS5 = 35.6% ($P < 0.001$; **Figure 2D**) and those using the criteria by Persiani et al. were LODDS1 = 88.2%, LODDS2 = 77.9%, LODDS3 = 53.6% ($P < 0.001$; **Figure 2E**). Significant survival differences were detected between the subgroups of each staging system (**Figure 2**,**Table 3**).

Prognostic Accuracy of Different LN Staging Systems

The AIC and c-index were used to estimate the prognostic discriminative ability of different LN staging systems (**Table 4**).

TABLE 2 | Clinical and histopathological characteristics for the entire population.

Variables	N (%)	Univariate analysis	
		5-year OS (%)	*P*-value
Age, years			<0.001
≤ 65	26,305 (46.4)	85.3	
> 65	30,442 (53.6)	72.6	
Gender			0.353
Male	27,507 (48.5)	78.5	
Female	29,240 (51.5)	78.7	
Tumor location			0.763
Rectum	10,613 (18.7)	78.3	
Colon	46,134 (81.3)	78.5	
Histologic grade			<0.001
Well differentiated	5,382 (9.5)	87.6	
Moderately differentiated	41,004 (72.3)	80.2	
Poorly differentiated	9,609 (16.9)	66.9	
Undifferentiated	752 (1.3)	65.4	
Tumor size, cm			<0.001
≤ 5	35,672 (62.9)	80.6	
> 5	16,259 (28.7)	72.2	
Unknown	4,816 (8.5)	85.6	
AJCC 8th T stage			<0.001
pT1	8,022 (14.1)	95.5	
pT2	9,957 (17.5)	91.6	
pT3	32,726 (57.7)	75.7	
pT4	6,042 (10.7)	51.6	
NDLN			<0.001
Inadequate ($n < 12$)	16,699 (29.4)	76.2	
Adequate ($n \geq 12$)	40,048 (70.6)	79.7	

N, number; OS, overall survival rate; NDLN, the number of dissected lymph nodes; AJCC, American Joint Committee on Cancer.

TABLE 1 | Basic characteristics of different lymph node staging systems.

AJCC 8th N stage (3)	LNR, Berger et al. (16)	LNR, Rosenberg et al. (17)	LODDS, Wang et al. (26)	LODDS, Persiani et al. (24)
pN0	LNR1 < 0.05	LNR0 = 0.00	LODDS1 < −2.2	LODDS1 ≤ −1.36
pN1a	0.05 ≤ LNR2 < 0.19	0.01 ≤ LNR1 ≤ 0.17	−2.2 ≤ LODDS2 < −1.1	−1.36 < LODDS2 ≤ −0.53
pN1b	0.19 ≤ LNR3 < 0.39	0.18 ≤ LNR2 ≤ 0.41	−1.1 ≤ LODDS3 < 0.0	LODDS3 > −0.53
pN2a	0.39 ≤ LNR4 ≤ 1.00	0.42 ≤ LNR3 ≤ 0.69	0.0 ≤ LODDS4 < 1.1	
pN2b		LNR4 ≥ 0.70	LODDS5 ≥ 1.1	

AJCC, American Joint Committee on Cancer; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes.

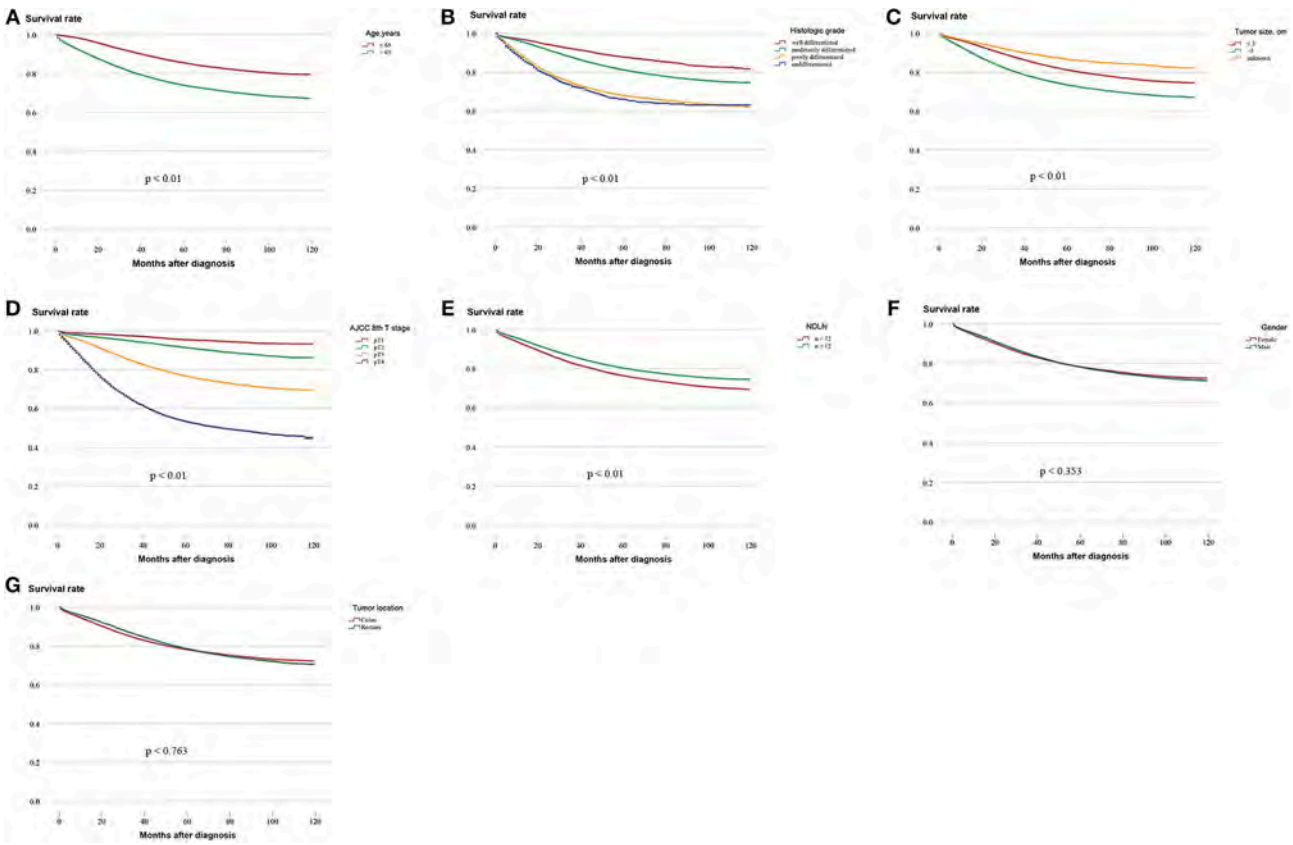

FIGURE 1 | Kaplan–Meier survival curves for five-year OS stratified by different prognostic factors with statistical significance based on the **(A)** Age, **(B)** Histologic grade, **(C)** Tumor size, **(D)** AJCC 8th T stage, **(E)** NDLN, **(F)** Gender, and **(G)** Tumor location. (AJCC, American Joint Committee on Cancer; NDLN, the number of dissected lymph nodes).

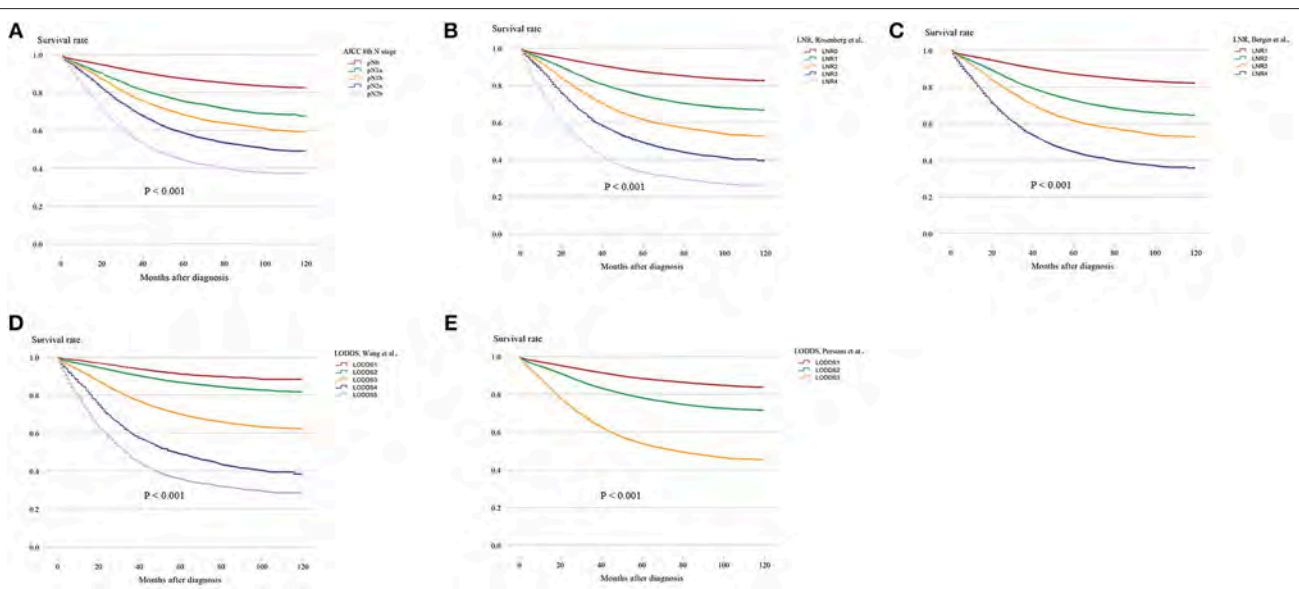

FIGURE 2 | Kaplan–Meier survival curves for five-year OS stratified by LN categories based on the **(A)** AJCC 8th N stage, **(B)** LNR of Rosenberg et al. **(C)** LNR of Berger et al. **(D)** LODDS of Wang et al. and **(E)** LODDS of Persiani et al. (LN, lymph node; AJCC, American Joint Committee on Cancer; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes).

TABLE 3 | Five-year overall survival and 95% confidence interval according to different LN staging.

Staging systems	N (%)	OR (95 % CI)	5-year OS (%)
AJCC 8th N stage (3)			
pN0	34,024 (60.0)	1.00 (Reference)	87.2
pN1a	6,975 (12.3)	2.06 (1.95–2.17)	75.2
pN1b	7,149 (12.6)	2.76 (2.63–2.90)	68.1
pN2a	4,764 (8.4)	3.82 (3.63–4.02)	58.3
pN2b	3,835 (6.8)	5.80 (5.52–6.10)	44.1
LNR, Berger et al. (16)			
LNR1	36,041 (63.5)	1.00 (Reference)	86.9
LNR2	10,058 (17.7)	2.26 (2.16–2.36)	72.4
LNR3	5,594 (9.9)	3.34 (3.19–3.51)	61.3
LNR4	5,054 (8.9)	5.62 (5.38–5.88)	44.3
LNR, Rosenberg et al. (17)			
LNR0	34,024 (60.0)	1.00 (Reference)	87.2
LNR1	11,520 (20.3)	2.41 (2.05–2.24)	74.1
LNR2	6,659 (11.7)	3.44 (3.29–3.61)	61.3
LNR3	2,919 (5.1)	5.06 (4.78–5.35)	48.9
LNR4	1,625 (2.9)	7.99 (7.49–8.53)	33.0
LODDS, Wang et al. (26)			
LODDS1	3,707 (6.5)	1.00 (Reference)	91.0
LODDS2	29,557 (52.1)	1.57 (1.41–1.75)	86.5
LODDS3	19,761 (34.8)	3.84 (3.45–4.27)	69.7
LODDS4	1,578 (2.8)	7.70 (6.80–8.70)	48.8
LODDS5	2,144 (3.8)	11.00 (9.80–12.35)	35.6
LODDS, Persiani et al. (24)			
LODDS1	24,983 (44.0)	1.00 (Reference)	88.2
LODDS2	21,423 (37.8)	1.97 (1.88–2.06)	77.9
LODDS3	10,341 (18.2)	4.81 (4.60–5.02)	53.6

N, number; OR, Odds Ratio; 95% CI, 95% confidence interval; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes; AJCC, American Joint Committee on Cancer.

First, the LN status was evaluated as a categorical variable to analyze the prognostic discriminating power of different LN staging systems. In the whole population, two LNR staging systems showed better prognostic performance than other staging systems, with the LNR from Rosenberg et al. (c-index: 0.669, AIC: 287984.1) showing the best prognostic performance. The LNR of Berger et al. (c-index: 0.666, AIC: 288125.3) and AJCC/UICC N staging (c-index: 0.666; AIC: 288397.0) had similar prognostic performances. In addition, the two LODDS (Wang et al.: c-index: 0.659, AIC: 288619.9; Persiani et al.: c-index: 0.659, AIC: 288994.6) staging systems performed relatively poorly. Further analysis based on different NDLN showed that when the NDLN was insufficient (NDLN < 12), the LNR of Rosenberg et al. (c-index: 0.649, AIC: 85842.9) still maintained the best prognostic performance. However, when the NDLN is sufficient (NDLN ≥ 12), AJCC/UICC N staging (c-index: 0.647; AIC: 85899.4) is the best prognostic model. In contrast, both LODDS staging systems showed the worst prognosis performance regardless of the adequacy of the NDLN.

To assess whether the ability of the predicted prognosis of different LN staging systems was affected by artificially determined cut-off values, the LN status was modeled as a continuous variable for repeated analysis. The results showed that the LODDS system was superior to other staging systems and was not affected by the NDLN. It is worth noting that PLN always showed the worst prognostic discriminative ability regardless of whether the NDLN was sufficient.

We created scatter plots to explain the relationship between LNR and LODDS. As shown in **Figure 3A**, when patients have different LNR, the LODDS has a one-to-one mapping value for each LNR, and as the LNR increases, the value of LODDS increases. This indicates a close correlation between LODDS and LNR (except when LNR $= 0$ or 1). Thus, both contain the same prognostic information. However, as shown in **Figures 3B,C**, when the LNR is close to 0 or 1, the value of LODDS is heterogeneous.

DISCUSSION

Regional LN metastasis of malignant tumors is one of the main metastatic patterns of CRC. LN status is also considered to be one of the most important prognostic parameters for recurrence and death after CRC resection. Therefore, accurate staging of LN status can more accurately predict cancer risk and lead to the development of postoperative treatment options for patients with CRC (16). A number of LN staging systems have been proposed to accurately describe LN status, including AJCC/UICC N staging, LNR, and the LODDS staging systems. Among them, the AJCC/UICC N staging system is widely recognized and used in clinical practice, but some scholars question its accuracy (19, 27–31). Some researchers have shown that the NPLN is significantly correlated with the NDLN, especially when the NDLN is insufficient, which may lead to the missed PLN, resulting in staging migration (6, 7, 16). LNR is a ratio-based LN status estimation method that considers both the NPLN and NPDLN. Many researchers have demonstrated that it is a better independent prognostic factor than the AJCC/UICC N staging in rectal cancer or colon cancer (27–31). Ozawa et al. studied the prognostic ability of LNR in stage IV CRC and found that patients with the same AJCC/UICC N staging group had 23% higher OS in the low LNR group than the high LNR group (32). This further illustrates that subgroups of patients with the same AJCC/UICC N stage can be divided into significantly different prognostic subgroups by the LNR system, and other studies have reached similar conclusions (17, 18). LODDS is another staging system that describes the LN status and has great potential to further improve the accuracy of LN staging for predicting prognosis. Persiani et al. used multivariate regression analysis to compare the accuracy of different LN staging systems in estimating the prognosis of colon cancer (24). That study demonstrated that LODDS is an independent prognostic factor, further showing that LODDS is more accurate than LNR in assessing colon cancer survival, and other researchers have used similar methods to draw similar conclusions (5, 21, 26, 33). However, they did not use statistical methods to directly compare the discriminative ability of different LN staging system models.

TABLE 4 | Prognostic performance of different lymph node staging systems before and after stratifying for NDLN.

Variables	NDLN					
	ALL (n = 56, 747)		≥ 12 (n = 16, 699)		< 12 (n = 40, 048)	
	C Index (95% CI)	AIC	C Index (95% CI)	AIC	C Index (95% CI)	AIC
PLN (continuous)	0.668 (0.663–0.672)	290576.3	0.682 (0.677–0.688)	186085.5	0.648 (0.641–0.655)	86189.8
LNR (continuous)	0.673 (0.668–0.677)	288763.7	0.684 (0.679–0.690)	185052.7	0.651 (0.644–0.658)	86050.7
LODDS (continuous)	0.682 (0.677–0.687)	287860.5	0.691 (0.685–0.697)	184338.2	0.652 (0.644–0.661)	85970.4
AJCC 8th N stage (3)	0.666 (0.662–0.671)	288397.0	0.681 (0.675–0.686)	184632.6	0.647 (0.640–0.654)	85899.4
LNR, Rosenberg et al. (17)	0.669 (0.664–0.673)	287984.1	0.679 (0.673–0.684)	184496.2	0.649 (0.642–0.656)	85842.9
LNR, Berger et al. (16)	0.666 (0.662–0.670)	288125.3	0.674 (0.669–0.679)	184686.9	0.639 (0.632–0.646)	85856.0
LODDS, Wang et al. (26)	0.659 (0.655–0.664)	288619.9	0.665 (0.660–0.670)	184888.2	0.629 (0.621–0.636)	86265.7
LODDS, Persiani et al. (24)	0.659 (0.654–0.663)	288994.6	0.673 (0.668–0.678)	184899.7	0.616 (0.609–0.623)	86388.1

NDLN, The number of dissected lymph nodes; AIC, Akaike's Information Criterion; C Index, Harrell's consistency Index; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes; AJCC, American Joint Committee on Cancer; PLN, positive lymph node.

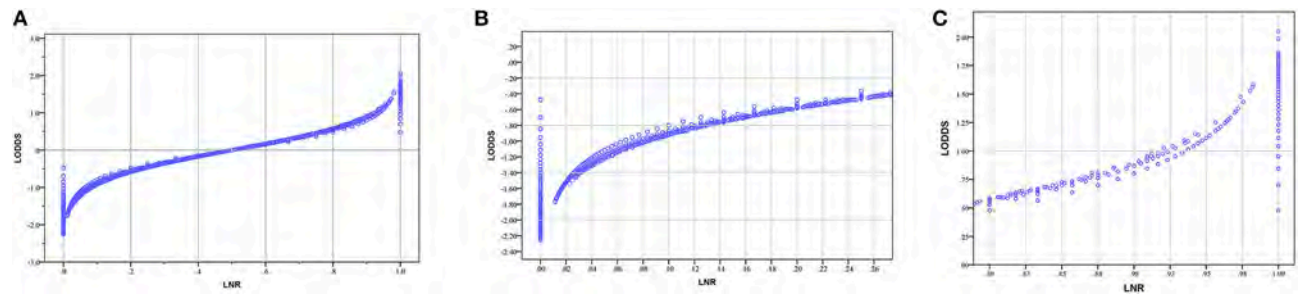

FIGURE 3 | (A) The scatter plots of LODDS vs. LNR; **(B)** The magnified view of **(A)** for LNR between 0 to 0.25; **(C)** The magnified view of A for LNR between 0.75 to 1. (LNR, lymph node ratio; LODDS, log odds of positive lymph nodes).

In our study, we used two statistical indicators, the AIC and the c-index, to analyze the relative discriminative ability of different LN staging systems in predicting CRC survival in a CRC patient population. We first analyzed LN status as a continuous variable. We found that LODDS is superior to PLN and LNR. When we analyzed LN status as a categorical variable, we showed that the two LNR staging systems were superior to other staging systems.

There is still controversy regarding the categorical cut-off values for different LN staging systems. The reason for heterogeneity in the cut-off values is multifactorial. First, different studies used different statistical methods to determine these optimal cut-off values. For example, Song et al. used log-rank statistical methods (34), Rosenberg et al. used categorical and regression tree techniques (17), Berger et al. used the quartile method (16), Kornprat et al. used the receiver operating characteristic (ROC) statistical method (35), and Wang et al. used the X-tile program (26). In addition, different countries and research institutions, differences in patient numbers, and different average NDLN also lead to the diversity in cut-off values.

In addition to LN status and categorical cut-off values, many studies have shown that the NDLN has a significant impact on patient prognosis. Le Voyer et al. showed that an increase in the NDLN was significantly associated with improved OS (7). The National Comprehensive Cancer Network (NCCN) guidelines recommend at least 12 NDLN for accurate staging. However, the NDLN in clinically resected specimens can vary greatly. In our study, the proportion of patients with insufficient NDLN reached 29.4%. In view of this, we conducted a subgroup study based on different NDLN to analyze the prognostic accuracy of each LN staging system. We divided patients into two subgroups according to the NDLN: NDLN < 12 and NDLN ≥ 12.

Therefore, we conducted a comprehensive study based on LN status (continuous variable and categorical variable) and the NDLN. When analyzed as a categorical variable, the LNR of Rosenberg et al. (17) was the best staging system when the NDLN <12. However, in patients with NDLN ≥ 12, AJCC/UICC N staging is the most accurate system for predicting patient outcomes. When analyzed as a continuous variable, LODDS showed the best discrimination ability regardless of the NDLN.

Many studies have shown that evaluating the LN status as a continuous variable reveals its true performance, so LODDS is a more accurate staging system than LNR in predicting CRC patient OS (36). We further illustrated the relationship between LNR and LODDS through scatter plots. **Figure 3** shows that the overall trend of LNR and LODDS is consistent. However, when the LNR is around 0 or 1, the value of LODDS is heterogeneous,

indicating that LODDS has a better discriminating power for patients with very low or high LNR. Some researchers believe that because of the lack of consensus on the cut-off values of different LN staging systems, LN status should be treated as a continuous variable (36). However, we believe that ignoring the cut-off values and using the LN status as a continuous variable cannot be applied in clinical practice. Thus, it has only theoretical value and no practical clinical value. Although LODDS is the best staging system, LODDS has no advantage over other staging systems when considering the impact of categorical cut-off values on staging systems. Therefore, optimal cut-off values should be calculated to make the LODDS staging system more useful for clinical practice.

The innovations of this study are as follows. First, the SEER data offers the unique opportunity to study prognostic elements in a larger number of patients. Second, in seeking the best staging system, we took the cut-off values of each staging system into account. However, there are limitations to our results, and we advise appropriate caution in their interpretation. This is a retrospective study based on the SEER database, so there will inevitably be some selection bias. The SEER database lacks some clinical information such as operative time, specific surgical procedures, lymph and/or vascular invasion, and specific locations of LN metastasis. Additionally, these results may not be applicable to other populations as they were based on Western patient data. Whether the use of this staging system could be applied to daily practice in Eastern countries, therefore, requires to be further validated. However, these shortcomings are common to any retrospective and population-based research. Finally, we believe that the patient data for this study is large and these shortcomings can be largely compensated by long-term follow-up.

CONCLUSIONS

In conclusion, we believe that regardless of the adequacy of the NDLN, LODDS is the most accurate staging system for predicting the survival of patients with CRC. However, the best LODDS cut-off values that can be applied to clinical practice have not been calculated. Therefore, the LNR staging system of Rosenberg et al. with cut-off values of 0.17, 0.41, and 0.69 should be introduced to the AJCC/UICC system as supplements when the NDLN are insufficient.

AUTHOR CONTRIBUTIONS

J-PP, C-DZ, and D-QD designed this study. J-PP and C-DZ performed search and collected data. Y-CF re-checked data. J-PP and C-DZ performed analysis. J-PP wrote the manuscript. C-DZ and D-QD reviewed the manuscript.

FUNDING

C-DZ was supported in part by Japan China Sasakawa Medical Fellowship.

ACKNOWLEDGMENTS

We acknowledge the efforts of the Surveillance, Epidemiology, and End Results (SEER) Program tumor registries for creating the SEER database. We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

REFERENCES

1. Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Rgs, M, Barzi A, et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin.* (2017) 67:104–17. doi: 10.3322/caac.21395
2. Compton CC, Greene FL. The staging of colorectal cancer: 2004 and beyond. *CA Cancer J Clin.* (2010) 54:295–308. doi: 10.3322/canjclin.54.6.295
3. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland, RK, et al. The eighth edition ajcc cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin.* (2017) 67:93–9. doi: 10.3322/caac.21388
4. Rice TW, Rusch VW, Ishwaran H, Blackstone EH. Cancer of the esophagus and esophagogastric junction. *Cancer* (2010) 116:3763–73. doi: 10.1002/cncr.25146
5. Arslan NC, Sokmen S, Canda AE, Terzi C, Sarioglu S. The prognostic impact of the log odds of positive lymph nodes in colon cancer. *Colorectal Dis.* (2015) 16:O386–92. doi: 10.1111/codi.12702
6. Tsai HL, Lu CY, Hsieh JS, Wu DC, Jan CM, Chai CY, et al. The prognostic significance of total lymph node harvest in patients with t 2–4 n 0 m 0, colorectal cancer. *J Gastrointest Surg.* (2007) 11:660–5. doi: 10.1007/s11605-007-0119-x
7. Le VT, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, et al. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial int-0089. *J Clin Oncol.* (2003) 21:2912–9. doi: 10.1200/JCO.2003.05.062
8. Kehoe J, Khatri VP. Staging and prognosis of colon cancer. *Surg Oncol Clin N Am.* (2006) 1:129–46. doi: 10.1016/j.soc.2005.08.006
9. Wong SL, Ji H, Hollenbeck BK, Morris AM, Baser O, Birkmeyer JD. Hospital lymph node examination rates and survival after resection for colon cancer. *J Am Med Assoc.* (2007) 298:2149–54. doi: 10.1001/jama.298.18.2149
10. Edler D, Ohrling K, Hallström M, Karlberg M, Ragnhammar P. The number of analyzed lymph nodes - a prognostic factor in colorectal cancer. *Acta Oncol.* (2007) 46:975–81. doi: 10.1080/02841860701203537
11. Walker R, Wood T, LeSouder E, Cleghorn M, Maganti M, MacNeill A, et al. Comparison of two novel staging systems with the tnm system in predicting stage iii colon cancer survival. *J Surg Oncol.* (2018) 117:1049–57. doi: 10.1002/jso.25009
12. Shen F, Cui J, Cai K, Pan H, Bu H, Yu F. Prognostic accuracy of different lymph node staging systems in rectal adenocarcinoma with or without preoperative radiation therapy. *Jpn J Clin Oncol.* (2018) 48: 625–32. doi: 10.1093/jjco/hyy070
13. Tamura M, Matsumoto I, Saito D, Yoshida S, Takata M, Takemura H. Lymph node ratio as a prognostic factor in patients with pathological n2 non-small cell lung cancer. *World J Surg Oncol.* (2016) 14:295. doi: 10.1186/s12957-016-1048-5
14. Ataseven B, Kümmel S, Weikel W, Heitz F, Holtschmidt J, Lorenz-Salehi F, et al. Additional prognostic value of lymph node ratio over pn staging in different breast cancer subtypes based on the results of 1,656 patients. *Arch Gynecol Obstet.* (2015) 291:1153–66. doi: 10.1007/s00404-014-3528-6
15. Smith DD, Nelson RA, Schwarz RE. A comparison of five competing lymph node staging schemes in a cohort of resectable gastric cancer patients. *Ann Surg Oncol.* (2014) 21:875–82. doi: 10.1245/s10434-013-3356-0
16. Berger AC, Sigurdson ER, Levoyer T, Hanlon A, Mayer RJ, Macdonald JS, et al. Colon cancer survival is associated with decreasing ratio of

metastatic to examined lymph nodes. *J Clin Oncol.* (2005) 23:8706–12. doi: 10.1200/JCO.2005.02.8852
17. Rosenberg R, Engel J, Bruns C, Heitland W, Hermes N, Jauch KW, et al. The prognostic value of lymph node ratio in a population-based collective of colorectal cancer patients. *Ann Surg.* (2010) 251:1070–8. doi: 10.1097/SLA.0b013e3181d7789d
18. Wang J, Hassett JM, Dayton MT, Kulaylat MN. Lymph node ratio: role in the staging of node-positive colon cancer. *Ann Surg Oncol.* (2008) 15:1600–8. doi: 10.1245/s10434-007-9716-x
19. Lee HY, Choi HJ, Park KJ, Shin JS, Kwon HC, Roh MS, et al. Prognostic significance of metastatic lymph node ratio in node-positive colon carcinoma. *Ann Surg Oncol.* (2007) 14:1712–7. doi: 10.1245/s10434-006-9322-3
20. Huang B, Chen C, Ni M, Mo S, Cai G, Cai S. Log odds of positive lymph nodes is a superior prognostic indicator in stage iii rectal cancer patients: a retrospective analysis of 17,632 patients in the seer database. *Int J Surg.* (2016) 32:24–30. doi: 10.1016/j.ijsu.2016.06.002
21. Lee CW, Wilkinson KH, Sheka AC, Leverson GE, Kennedy GD. The log odds of positive lymph nodes stratifies and predicts survival of high-risk individuals among stage iii rectal cancer patients. *Oncologist* (2016) 21:425–32. doi: 10.1634/theoncologist.2015-0441
22. Sun Z, Xu Y, Li de M, Wang ZN, Zhu GL, Huang BJ, et al. Log odds of positive lymph nodes: a novel prognostic indicator superior to the number-based and the ratio-based n category for gastric cancer patients with r0 resection. *Cancer* (2010) 116:2571–80. doi: 10.1002/cncr.24989
23. Chen LJ, Chung KP, Chang YJ, Chang YJ. Ratio and log odds of positive lymph nodes in breast cancer patients with mastectomy. *Surg Oncol.* (2015) 24:239–47. doi: 10.1016/j.suronc.2015.05.001
24. Persiani R, Cananzi FCM, Biondi A, Paliani G, Tufo A, Ferrara F, et al. Log odds of positive lymph nodes in colon cancer: a meaningful ratio-based lymph node classification system. *World J Surg.* (2012) 36:667–74. doi: 10.1007/s00268-011-1415-x
25. La TM, Nigri G, Petrucciani N, Cavallini M, Aurello P, Cosenza G, et al. Prognostic assessment of different lymph node staging methods for pancreatic cancer with r0 resection: pn staging, lymph node ratio, log odds of positive lymph nodes. *Pancreatology* (2014) 14:289–94. doi: 10.1016/j.pan.2014.05.794
26. Wang J, Hassett JM, Dayton MT, Kulaylat MN. The prognostic superiority of log odds of positive lymph nodes in stage iii colon cancer. *J Gastrointest Surg.* (2008) 12:1790–6. doi: 10.1007/s11605-008-0651-3
27. In Ja P, Sik YC, Seok-Byung L, Sik YY, Wook KC, Tae Won K, et al. Ratio of metastatic lymph nodes is more important for rectal cancer patients treated with preoperative chemoradiotherapy. *World J Gastroenterol.* (2015) 21:3274–81. doi: 10.3748/wjg.v21.i11.3274
28. Leonard D, Remue C, Orabi NA, Maanen AV, Danse E, Dragean A, et al. Lymph node ratio and surgical quality are strong prognostic factors of rectal cancer: results from a single referral centre. *Colorectal Dis.* (2016) 18:O175–84. doi: 10.1111/codi.13362
29. Lykke J, Jess P, Roikjaer O, Group DCC. The prognostic value of lymph node ratio in a national cohort of rectal cancer patients. *Eur J Surg Oncol.* (2016) 42:504–12. doi: 10.1016/j.ejso.2016.01.012
30. Zhou D, Ye M, Bai Y, Rong L, Hou Y. Prognostic value of lymph node ratio in survival of patients with locally advanced rectal cancer. *Can J Surg J.* (2015) 58:237–44. doi: 10.1503/cjs.001515
31. Peschaud F, Benoist S, Julié C, Beauchet A, Penna C, Rougier P, et al. The ratio of metastatic to examined lymph nodes is a powerful independent prognostic factor in rectal cancer. *Ann Surg.* (2008) 248:1067–73. doi: 10.1097/SLA.0b013e31818842ec
32. Ozawa T, Ishihara S, Nishikawa T, Tanaka T, Tanaka J, Kiyomatsu T, et al. Prognostic significance of the lymph node ratio in stage iv colorectal cancer patients who have undergone curative resection. *Ann Surg Oncol.* (2015) 22:1513–9. doi: 10.1245/s10434-014-4184-6
33. Scarinci A, Di TC, Cavaniglia D, Neri T, Colletti M, Cosenza G, et al. The impact of log odds of positive lymph nodes (lodds) in colon and rectal cancer patient stratification: a single-center analysis of 323 patients. *Updates Surg.* (2018) 70:23–31. doi: 10.1007/s13304-018-0519-3
34. Song YX, Gao P, Wang ZN, Tong LL, Xu YY, Sun Z, et al. Which is the most suitable classification for colorectal cancer, log odds, the number or the ratio of positive lymph nodes? *PLoS ONE* (2011) 6:e28937. doi: 10.1371/journal.pone.0028937
35. Kornprat P, Pollheimer MJ, Lindtner RA, Schlemmer A, Rehak P, Langner C. Value of tumor size as a prognostic variable in colorectal cancer: a critical reappraisal. *Am J Clin Oncol.* (2011) 34:43–9. doi: 10.1097/COC.0b013e3181cae8dd
36. Spolverato G, Ejaz A, Kim Y, Squires MH, Poultsides G, Fields RC, et al. Prognostic performance of different lymph node staging systems after curative intent resection for gastric adenocarcinoma. *Ann Surg.* (2015) 262:991–8. doi: 10.1097/SLA.0000000000001040

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.