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Letters to the Editor

Six amino acids of VP1 switch along with pandemic of CV-A6-associated HFMD in Guangxi, southern China, 2010–2017

Dear Editor,

We read with interest a recent article in this journal that the predominant pathogen shift to CV-A6 might contribute to the abnormal growth of Hand, foot, and mouth disease (HFMD) incidence in Guangdong province of China in autumn of 2017.[1] HFMD is a common childhood infectious disease, is a global public health issue, especially in the Asia-Pacific region. The symptoms of HFMD are generally mild, which include fever, blisters on the hands and feet, and ulcers inside or around the mouth. However, some infections developed serious neurological or systemic complications, even fatal. Enterovirus 71 (EV-A71) and coxsackievirus A16 (CV-A16) were the primary HFMD pathogens before 2013 and CV-A6 has become a major HFMD pathogen since 2013. The Guangxi autonomous region in southern China has been one of the top three provinces most affected by HFMD in China since 2008.[2] In Guangxi, the HFMD cases in Qinzhou raised dramatically in 2016 and 2017. However, the characteristics of pathogen spectrum are still unknown. This study focused on characterizing molecular evolution of CV-A6 in Qinzhou, from January 2010 to December 2017.

Mild cases of HFMD are defined as the absence or presence of fever accompanied by a rash (maculopapule or vesicular rash) appearing on either the hand, foot, mouth, or buttock. Severe cases are defined as the presence of at least one of the following complications: acute flaccid paralysis, aseptic meningitis, pulmonary edema, encephalitis, hemorrhage, and cardiopulmonary collapse. Clinical specimens (stool, rectal swabs or throat swabs) were collected and then Enterovirus were detected. All analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/).

The Guangxi autonomous region is located in southern China and Qinzhou city is a coastal city (Fig. S1A and B). In this study, a total of 45,169 clinical cases, with 1344 severe cases and 20 fatal cases, were reported in Qinzhou from January 2010 to December 2017. Generally, a periodical cycle with one-year interval of HFMD outbreak was observed in Guangxi from 2010 to 2016. Outbreak was persistent in 2017 even after the large scale of outbreak in 2016, that was once predicted to be a small epidemics year according to the empirical period of outbreak. Compared to those in years with sporadic prevalence (2011, 2013, and 2015), more cases were involved in years with outbreaks (including 2010, 2012, 2014, 2016 and 2017) (Fig. S1C). Higher proportion (3.51%, 4.68%, and 5.47%) of severe cases were reported in 2012, 2014, 2016, compared with those in 2010, 2011, 2013, 2015, and 2017 (0.95%, 0.05%, 0.31%, 2.44%, and 2.37%).

The HFMD cases occurred all the year round with the peaks during April to July (the summer peak) or August to November (the autumn peak) (Fig. 1A). Double peaks of pandemic were observed in 2010, 2014 and 2017 respectively, only a single peak in summer was occurred in 2012 and 2016, while only a single peak in autumn was taken place in 2013 and 2015. Most severe cases were occurred in summer (Fig. 1B). For the first time, we observed that the number of cases in autumn season was higher than the number in summer compared to other years with outbreaks (2010, 2012, 2014, and 2016), and reached the historical top.

RNA extraction and detection of pathogens (pan-EV, EV-A71, CV-A16, C-A6 and CV-A10) detection was performed as previously described.[3] Among 3251 HFMD samples, a total of 1990 HFMD cases were positive for enteroviruses, including 1102 cases (55.38%) positive for EV-A71, 289 (14.52%) positive for CV-A16, 460 (18.02%) positive for CV-A6, and 59 (2.31%) positive for CV-A10. In general, EV-A71, CV-A6 and CV-A16 are the major pathogens in Qinzhou during 2010-2017 (Fig. S1E). The number of mild HFMD cases with positive for enteroviruses during 2010-2017, were 48, 123, 158, 97, 173,165, 204, and 110, respectively. For mild cases, EV-A71, CV-A6 and CV-A16 were the major pathogens. It is noticeable that CV-A6 positive rate increased sharply in 2015 and 2017, up to 77.58% (128/165) and 49.09% (54/110), respectively (Fig. 1C). For severe cases, although EV71 was still the dominant pathogen, CV-A6 positive rate increased significantly in 2015 and 2017 to 30.34% and 36.72% respectively (Fig. 1D). Thus, CV-A6 positive rate increased both in mild cases and severe cases in 2015 and 2017.

In summer, all of EV-A71, CV-A6 and CV-A16 present substantially (Fig. 1E). Meanwhile, in autumn, CV-A6 has been the major, even dominant pathogen since 2011 (Fig. 1F). Overall, EV71 is dominant in 2010, 2012, 2014, and 2016, while CV-A6 is dominant in 2015 and 2017 (Fig. S1E).

The complete VP1 gene sequences of 85 CV-A6 positive samples were amplified and sequenced as previously described.[3,4] Then the 85 VP1 sequences were submitted to GenBank (Accession numbers: MF185255 to 185311). Evolutionary history was inferred using the neighbor-joining method.[5] Phylogenetic analysis of complete VP1 sequences (915 nucleotides) of 175 global CV-A6 strains, including those isolated from 46 mild cases and 39 severe cases in Qinzhou, indicated that the strains could be divided into four genetic subgroups, genotypes A, B, C and D. The stains in mainland of China during 2008–2015 falled into D2 and D3.[6] All of the CV-A6 strains identified in this study exhibited 93.8–99.8% nucleotide similarity, 97.0–100.0% amino acid similarity correspondingly, between each other. All of these 85 strains were clustered exclusively to genotype D, subtype 3a (Fig. 2A), the dominant subtype circulating in mainland China in the recent years. Our stains were clustered with stains isolated from other provinces of China.

Then, the signatures of VP1 amino acids were investigated and six amino acids were found nonconservative. The heatmap of these six sites showed that six amino acids of 85 CV-A6 falled into three branches: 2010–2012, 2013–2015 and 2016–2017 (Fig. 2B). The V174I and T283A may happen in 2016, while the switches of

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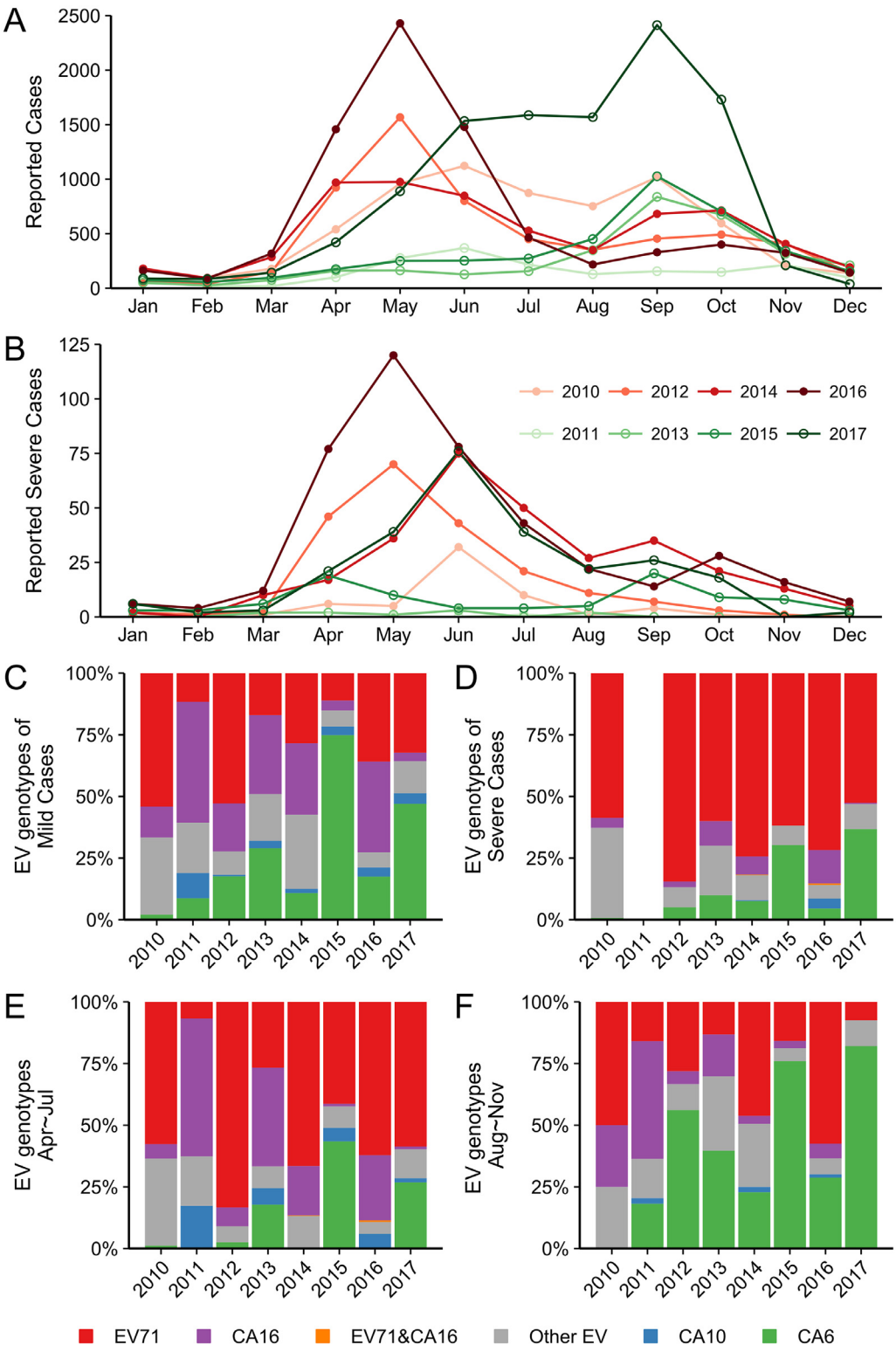

Fig. 1. Monthly distribution of reported cases (A) and severe cases (B) of hand, foot, and mouth disease (HFMD) in Qinzhou during 2010–2017. The EV genotypes of mild cases (C) and severe cases (D) of HFMD in Qinzhou during 2010–2017. The EV genotypes of summer peaks (E) and the autumn peaks (F) of HFMD cases in Qinzhou during 2010–2017.

the left four sites may occur during 2013-2014. Interestingly, some sites tend to switch bivalently, V174I and T283A, S137N and I242V, A5T and V30A.

Previous study showed that CV-A6 contain both non-lethal and lethal styles in the mouse model.[7] Interestingly, the amino acids of the four switches, A5T, V30A, S137N and I242V, of most strains isolated during 2010–2012 in our study were the same to the non-lethal stains. In contrast, these four sites of CV-A6 isolated during 2013–2015 and 2016–2017 were the same with those of the lethal stains. Thus, CV-A6 may have become more virulent and contributed to more mild and severe cases in Guangxi since 2013. These four sites swiches occurred in both mild and severe

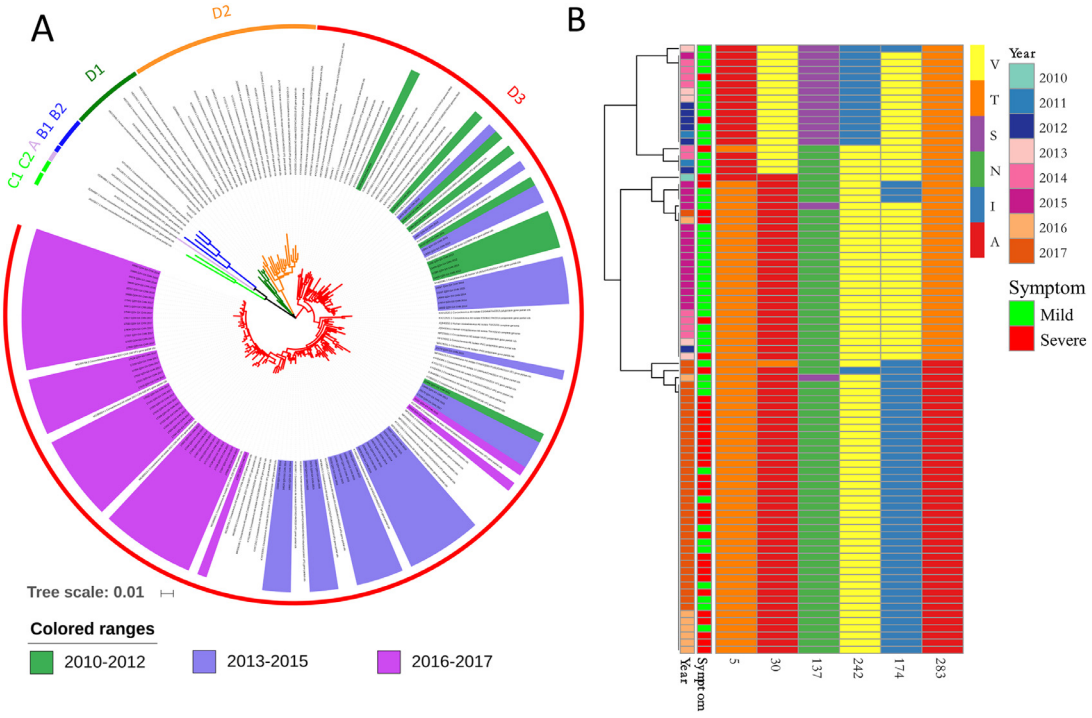

Fig. 2. (A) Phylogenetic tree was constructed from the 915 bp VP1 nucleotide sequences of CV-A6 using neighbor-joining method with 1000 bootstrap by MEGA7. It was displayed using the online tool, Interactive Tree of Life (iTOL). (B) Heatmap of the distribution for six amino acid sites of 85 CV-A6 in Qinzhou isolated during 2010–2017. It was created using pheatmap in R.

HFMD cases, so there are still need further study to validate the relationship of these four amino acids switches and the virulence of CV-A6.

The phenomenon also exists in Vietnam. CV-A6 has become an emerging pathogen in Vietnam since 2011. The four switching sites, A5T, V30A, S137N and I242V, also occurs in the circulating CV-A6 stains causing HFMD in Vietnam from 2011 to 2015[8] with consistent lethal properties. Interestingly, the structure of 137N is a bend[9] and the switching of S137N will add a new N-glycosylation site, but the functions still remain to be studied.

There were eight candidate variations are identified from the CV-A6 stains in Xiamen of China.[10] However, the evolution model in our study are different from that of Xiamen, suggesting that the circulating CV-A6 as well as its evolution pattern are geographical different.

Overall, the prevalence of EV-A71 and CV-A6 oscillates following big and small epidemic years. EV71 is the primary pathogen for HFMD in summer, while CV-A6 is dominant in autumn. our results indicates that six amino acid switches, V174I, T283A,CV-A6 VP1, A5T, V30A, S137N, and I242V, circulating in Guangxi during 2010–2017 may be associated with CV-A6 pandemic. These results help to explain the prevalence of CV-A6 and the epidemic characteristic of HFMD in Guangxi, southern China.

Conflicts of interest

The authors declared that they have no conflicts of interest to this work.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2019.02.002.

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Invasive meningococcal disease as a cause of sudden and unexpected death in a teenager: The public health importance of confirming the diagnosis

Dear Editor,

We read with interest the article in this Journal by Hong et al., who describe the clonal replacement and expansion of a hypervirulent strain of group W *Neisseria meningitidis* (MenW) belonging to the ST11 clonal complex (cc11) in France, which was associated with a high case fatality rate (CFR)[1]. Invasive meningococcal disease (IMD) is notorious for its sudden onset and rapid progression to life-threatening infection and death[2]. In adolescents and young adults, where the condition can mimic other illnesses, such as influenza, food poisoning, drug misuse or alcohol intoxication, the diagnosis of IMD may not be considered by clinicians in the acute stages of the illness. Where IMD is suspected, antibiotic administration prior to hospital transfer or prior to performing blood cultures increases the likelihood of negative bacterial cultures and, therefore, missing the aetiology[3]. The diagnosis of IMD might also be missed in cases with a rapidly progressing illness resulting in sudden death outside the hospital setting, where the condition of the patient prior to death might not have been witnessed. We were recently involved with such a case, who was diagnosed with IMD four months after death, and would like to highlight the clinical and public health importance of ascertaining this rare but important diagnosis, even at post-mortem.

An 18-year old first-year university student developed mild headache on a Friday, which worsened on Saturday although the case was well enough to attend a concert that evening. The case returned to the family home around midday Sunday, ate lunch and slept in the afternoon, but woke up reporting cold hands and feet at 18:00 and stomach ache with vomiting at 23:00, 02:00 and 04:00. The case's parents contacted the out-of-hours doctor and a clinical diagnosis of acute food-poisoning following chicken consumption at the concert was made during the telephone consultation. A plan was made for medical review at the case's home, which occurred at 05:30. At this point, the case was seriously unwell. Intramuscular benzylpenicillin was administered immediately and the case was transferred by ambulance to the hospital. The case developed septic shock, continued to deteriorate rapidly and passed away at 10:00 on the same day.

A post-mortem was performed four days later but only toxicology samples were taken for analysis. Three months later, the coroner reported the cause of death as unascertained. Despite the clinical history being consistent with serious bacterial infection, a drug-related cause of death was suspected because of the age of the case. It was only after the parents contacted a national meningitis charity that it became apparent that testing for meningococcal disease (or any other infectious disease) had not been undertaken at the hospital or during post-mortem investigation. After extensive discussions with the coroner, four stored post-mortem blood samples were retrieved from the local hospital and sent to the Public Health England (PHE) Meningococcal Reference Unit (MRU) for PCR-testing. A diagnosis of group W IMD was confirmed within two days of sample receipt.

Infection is known to be a rare cause of death in healthy teenagers and young adults[4], in contrast to drug use and drug dependence, which are recognised as common causes of early death. Death due to drug misuse occurs when the underlying cause is (a) drug abuse or drug dependence; or (b) drug poisoning and where any of the substances controlled under the Misuse of Drugs Act 1971 are involved[5]. In England and Wales, drug poisoning was responsible for 1 in 6 deaths among people in their 20 s and 30 s in 2016[5]. During 2016, there were 2153 recorded deaths in 15–24 year-olds (1487 male, 69%) and 1244 (966 male, 78%) were due to 'External causes of morbidity and mortality'[6].

Death due to IMD is rare, especially in teenagers. In England, during 2014/15-2016/17, there were 368 IMD cases (138 cases in 2016/17; incidence, 1.8/100,000) and 23 deaths recorded on the death certificates of 15–24 year-olds (CFR 6%, equivalent to an annual mortality rate of ~1 per million). Additionally, enhanced national IMD surveillance through PHE identified only four additional IMD-associated deaths in 15–24 year olds over the same three-year period.

Whilst recognising that drug use and drug dependence are leading causes of death in teenagers and young adults, it is important to consider an infectious cause, especially when infection-related symptoms precede the death, even if there is a history of social activity or behaviour which may increase suspicion for drug misuse, such as clubbing, recent recreational drug taking or alcohol consumption, which is common in this age-group. New university entrants, especially those in halls of residence, have a > 10-fold higher risk of developing IMD compared to those in the same age-group who are not attending university[7].

Early recognition of IMD cases is important:

- So that families are appropriately and accurately informed without undue delay.
- To ensure timely and appropriate public health actions to minimise the short-term and long-term risk in close contacts of the case, including rapid antibiotic chemoprophylaxis and vaccination where appropriate;

- To facilitate monitoring of outbreaks through disease surveillance; timely identification of an outbreak could potentially lead to implementation of mass chemoprophylaxis with or without vaccination to prevent further cases from occurring.
- To ensure that any specific characteristics of circulating strains are identified so that clinicians can be informed, such as group W meningococcal disease and gastrointestinal presentation[8].

Whilst drug misuse remains the most important cause of sudden and unexpected death in teenagers and young adults, other causes, especially those related to infection, should be considered. In particular, confirming IMD is critical because of potential clinical and public health implications for the family and the wider community. Since administering antibiotics prior to taking microbiological specimens is likely to yield negative bacterial cultures, additional investigations such as molecular testing, including PCR-testing for IMD and other infectious diseases, should be routinely performed in all fatal cases where the cause of death is not ascertained.

Consent for publication

Written informed consent was obtained from the parent of this patient for publication of the case report.

Funding

None.

Competing interests

HC, CA, MR and SNL have no personal competing interest. MR is a medical advisor and on the research panel for the MRF and Meningitis Now charities respectively. PHE Immunisation and Countermeasures Division has provided vaccine manufactures with post-marketing surveillance reports which the companies are required to submit to the UK Licensing authority in compliance with their Risk Management Strategy. In accordance with PHE policy a cost recovery charge is made for these reports. RB performs contract research on behalf of Public Health England for GSK, Pfizer and Sanofi Pasteur.

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We would like to thank the parents of this case for sharing their experiences and Steve Dayman of Meningitis Now for his support in generating this case report. Meningitis Now and the Meningococcal Research Foundation were very keen to highlight the importance of missed diagnosis of IMD even at post mortem in the light of the unnecessary pain that this has caused some families.

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Diagnosing urinary tract infection in older people

To the Editor,

The recently published study by Gbinigie et al. highlights both the importance and difficulty with the diagnosis of urinary tract infection (UTI) in older people.[1] They have performed a thorough analysis of available studies in this field. However, any meta-analysis is limited in worth by the quality of the studies that it contains. The authors have appropriately acknowledged the high rate of asymptomatic bacteriuria (ASB) in older people, yet the majority of the included studies used the presence of bacteriuria alone as a diagnostic standard for UTI. It is well known that ASB is commoner in older and frailer people and should not be treated with antibiotics.[2] Many of the studies recruited care home residents, in whom the prevalence of ASB is likely to be in the range of 15–50%.[3] The associations generated failed to strongly link urinary tract symptoms to a diagnosis of UTI, which further suggests that ASB was being mislabelled as UTI within the included studies. Previous data have also not associated urinary tract symptoms with bacteriuria alone.[4] In addition, signs traditionally associated with sepsis (pyrexia, hypotension and tachycardia) were not associated with the label 'UTI' in this analysis.

Given the lack of diagnostic gold standard for UTI, it is an area that is very difficult to effectively study. People with bacteraemic UTI (i.e. blood and urine cultures simultaneously positive for a likely uropathogen without an obvious alternative reason) are very likely to have a genuine UTI and are at the highest risk of mortality. A study performed in this group found that 92% of people aged over 75 had at least mild pyrexia and 60% had tachycardia.[5] In addition, 51% had at least one urinary tract symptom, which is consistent with 47% having symptoms when diagnosed with UTI by expert consensus in an emergency department.[6] In the latter group, non-specific signs such as lethargy and altered mental status were poor predictors for bacterial infection. Gbinigie et al. also found an association with a label of UTI and foul smelling urine in men,[1] but change in urine character is non-specific and has other potential causes such as dehydration or misrecognition of a reduced ability to self-perform personal care.

UTI has a high misdiagnosis rate in older people with a tendency to over-diagnose.[7] This leads to inappropriate antibiotic exposure and failure to identify the correct reason for deterioration in a timely way. The authors have attempted to use an analysis of studies to address this problem but the studies they identified are flawed, which has led them to false conclusions. They have effectively demonstrated the known association between old age, frailty (with reduced functional ability) and the finding of ASB, which is not the same as having a UTI. This could serve to reinforce the erroneous assumption that non-specific illness is frequently due to UTI in older people, resulting in harm from over exposure to antibiotics and missing the real problem. It has also led them to overlook the genuine association between urinary tract symptoms and symptomatic UTI, by definition, and the systemic signs of sepsis associated with bacteraemic UTI. This could put patients at risk of harm from under treatment. It is my opinion that the authors should simply have described the limitations with the currently available data in this field rather than draw potentially harmful conclusions.

Conflict of interest

I have no conflicts of interest.

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The clinical significance of thrombocytopenia complicating sepsis: A meta-analysis

Dear Editor,

We read with interest the recent review by Ronald Anderson et al.[1] that addressed an overview of the limited experimental studies together with a larger series of clinical studies mainly focused on all-cause CAP, which have provided evidence in support of associations between alterations in circulating platelet counts, most commonly thrombocytopenia, and a poor clinical outcome. The final section of the review covers, albeit briefly, systemic biomarkers of platelet activation which may have prognostic potential. This article reports a meta-analysis and systematic review of studies of thrombocytopaenia in sepsis compared to no thrombocytopaenia in sepsis. A systematic review of published work was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.[2] A systematic search of the PubMed, MEDLINE, Cochrane Library and Wanfang databases was performed from January 2008 to January 2018, using the following terms to identify studies reporting the outcomes of thrombocytopaenic and non-thrombocytopaenic adults with sepsis: “sepsis,” “thrombocytopenia,” and “mortality”. The searches were limited to articles published in the English and Chinese languages. Further articles were identified by hand-searching the reference lists of all retrieved articles to identify potentially relevant studies. Searches were cross-referenced on PubMed using the related articles function. The last search date was 31 January 2018. The primary endpoint of this meta-analysis was the incidence of overall hospital mortality. The secondary endpoint was the incidence of complications. When there were multiple articles by the same authors that analysed data from the same or a similar patient group, the most recent publication was included if the study periods overlapped. Review articles, case reports, experimental studies and studies that did not report outcomes were excluded. Unpublished data from conference abstracts were excluded. Only studies with more than five patients were included.

The characteristics of the included trials are listed in Table 1. Finally, data from 16 articles were included: 12 articles from the English-language literature and 4 articles from the Chinese-language literature. Of these articles, 12 were cohort studies and

Abbreviations: DIC, disseminated intravascular coagulopathy; ICU, Intensive Care Unit; AKI, acute renal injury; ARDS, adult respiratory distress syndrome.

Table 1
Characteristics of the included studies (according to the Cochrane Collaboration's tool for assessing risk of bias).

Ref.	Country	Number of centre	Study Type	ICU Type	Sample sizes	Population	Incomplete outcome	Selective outcome	Other sources of bias
Bedet et al [1]2017	France	1	Pro	Mixed	60	septic shock	No missing outcome data	All prespecifiedoutcomes reported	no
Boechat et al[2]2012	Brasil	1	Retr	NR	56	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Burunsuzoglu et al [3]2016	Turkey	1	Retr	NR	307	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Claushuis et al[4] 2016	Netherlands	1	Pro	Mixed	929	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Cui et al[5]2016	China	1	Retr	NR	60	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Gafter-Gvili et al[6]2011	Israel	1	Retr	NR	1052	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Hou et al[7] 2016	China	1	Retr	Emergency	78	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Koyama et al[8] 2018	Japan	1	Retr	medico-surgical	205	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Lim et al[9] 2012	Korean	1	Pro	medical	186	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Martin et al[10] 2009	Canadian	12	Pro	Mixed	1238	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Semeraro et al[11]2017	Italy	40	Retr	NR	280	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Sun et al[12] 2014	China	1	Retr	NR	95	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Thiery-antier et al[13] 2016	France	14	Retr	NR	1486	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Tsirigotis et al[14]2016	Greece	1	Retr	NR	105	Sepsis/septic shock	No missing outcome data	All prespecifiedoutcomes reported	no
Vandijck et al [15]2010	Belgium	1	Retro	NR	155	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no
Venkata et al[16] 2013	Mayo Clinic Medical Center, USA	1	Retro	medical	304	severe sepsis/septic shock	No missing outcome data	All prespecifiedoutcomes reported	no
Wang et al[17] 2015	China	1	Retr	NR	80	Sepsis	No missing outcome data	All prespecifiedoutcomes reported	no

1 was a case-control study. The thrombocytopaenia group contained 1191 patients, and the non-thrombocytopaenia group contained 1323 patients.

Seventeen studies reported that thrombocytopaenia increased overall mortality. Because the heterogeneity was too large, we chose thirteen studies with minimum heterogeneity. The heterogeneity among these studies was significant ($P < 0.00001$, $I^2 = 76\%$, Fig. 1A). Thus, we used the random-effects model and found that there was a significant difference in mortality between the thrombocytopaenia and non-thrombocytopaenia groups (OR = 3.14, 95% CI: 2.36-4.17, P < 0.00001, Fig. 1A). The sensitivity analysis, which was performed by removing each study in turn from the overall data or by restricting the meta-analysis to high-quality studies, showed that the result was robust.

Nine studies evaluated the occurrence of shock in both groups. These trials were significantly heterogeneous (P < 0.0001, $I^2 = 77\%$), and a random-effects analysis was performed. The statistical results showed that thrombocytopaenia was associated with an increased incidence of shock (OR = 2.4, 95% CI: 1.49-3.85, $P = 0.0003$, Fig. 1B), and the result did not change by restricting the meta-analysis to the five high-quality studies (OR = 3.14, 95% CI: 1.95-5.06, $P < 0.0001$).

Only three studies reported bleeding events in sepsis, including spontaneous bleeding in organs such as the gut, lungs, brain and so on. These trials were not significantly heterogeneous (P = 0.99, $I^2 = 0\%$), and a random-effects analysis was performed. The meta-analysis showed a significant difference between the thrombocytopaenia and non-thrombocytopaenia groups (OR = 4.44, 95% CI: 2.04-9.69, $P = 0.0002$).

Five studies reported AKI in sepsis. These trials were not significantly heterogeneous (P = 0.11, $I^2 = 47\%$), and a random-effects analysis was performed. The meta-analysis showed a significant difference between the thrombocytopaenia and non-thrombocytopaenia groups (OR = 2.19, 95% CI: 1.77-2.71, $P < 0.0001$, Fig. 1C).

Seven studies reported the use of mechanical ventilation during hospitalization. These trials were not significantly heterogeneous (P = 0.79, $I^2 = 0\%$), and a random-effects analysis was performed. The results indicated no significantly increased dependence on mechanical ventilation in the thrombocytopaenia group (OR = 1.12, 95% CI: 0.93-1.35, $P = 0.24$).Only three studies reported ARDS in sepsis. These trials were not significantly heterogeneous (P = 0.46, $I^2 = 0\%$), and a random-effects analysis was performed. The meta-analysis showed no difference between the thrombocytopaenia and non-thrombocytopaenia groups (OR = 1.42, 95% CI: 0.96-2.09, $P = 0.08$).

The publication bias was assessed using a funnel plot (Fig. 2). The plot shows that the overall distribution of the studies included was symmetrical, and no obvious bias was found.

The aim of the present work was to perform a meta-analysis on thrombocytopaenia in sepsic patients and its association with various prognostic value. We found that thrombocytopaenia is associated with poor prognosis and increases the complication rate in sepsis. Theoretically, the number and function of platelets are related to the incidence of many diseases. The involvement of platelets in inflammation and coagulation reactions, especially in the occurrence and development of sepsis, indicates that they play a key role in the transformation to sepsis.[3] Studies have shown

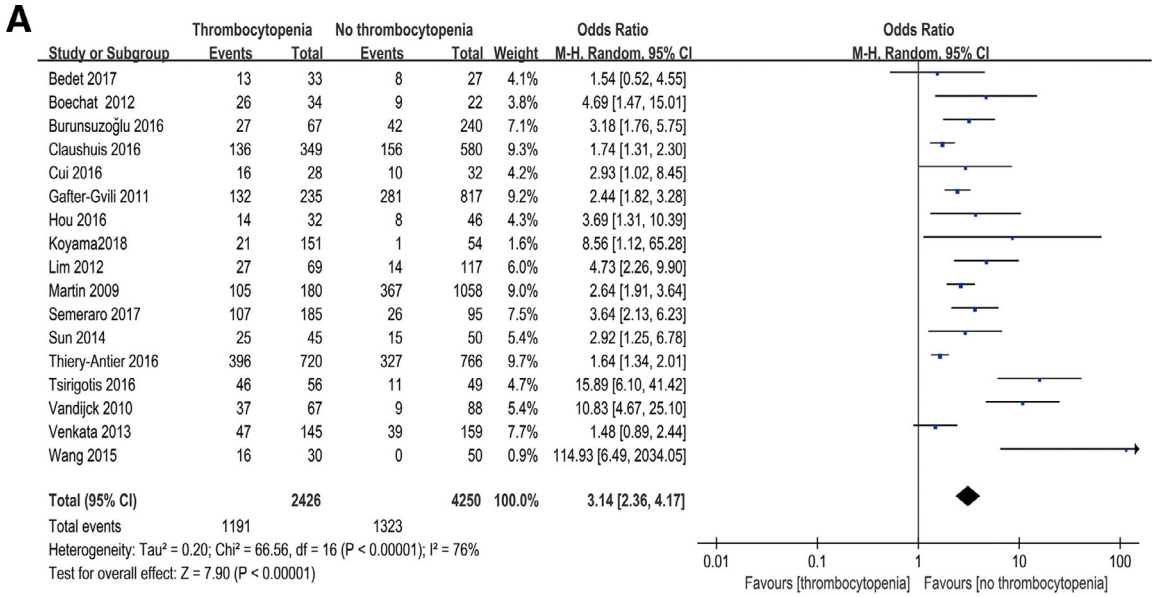

Fig. 1. Forest plot demonstrating significant difference in A mortality, B shock and C AKI between thrombocytopenia and without thrombocytopenia in sepsis.

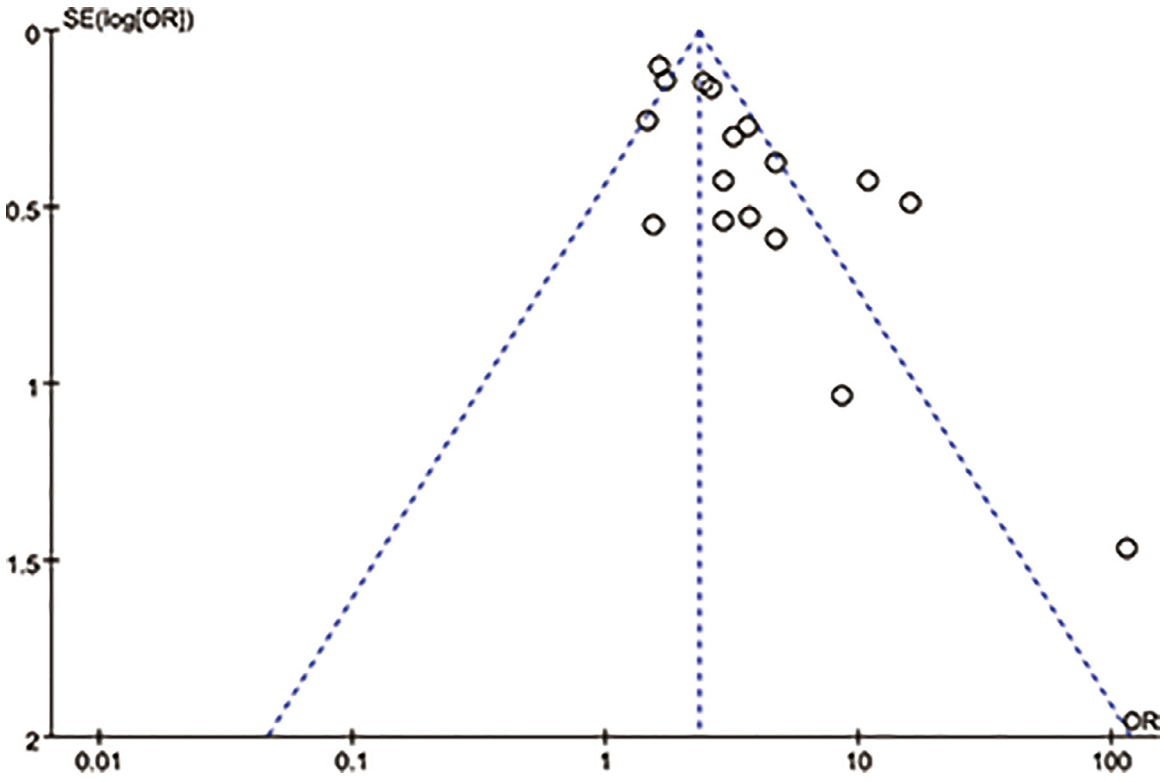

Fig. 2. Funnel plot did not show publication bias.

that thrombocytopaenia may be present in patients with early-stage sepsis. A study in 2002 by Strauss et al.[4] noted that platelet count reduction is one of the independent factors for death in ICU patients.

Therefore, a decrease in platelet count can be used to indicate the severity of sepsis, and trends in platelet count changes can be used to judge the recovery and prognosis of patients with sepsis. Combining platelet count with the clinical score in evaluating the severity and prognosis of the disease may lead to a more accurate prediction of the recovery or deteriorating clinical status of patients with sepsis.

As numerous studies have shown, platelet-related parameters may represent the degree of inflammatory reaction, platelet activity and compensatory bone marrow hyperplasia in sepsis patients and can be indirectly representative of the severity of the disease.[2,4] This study was conducted by observing the relationship between thrombocytopaenia and mortality in sepsis. Our meta-analysis suggested that thrombocytopaenia was associated with higher mortality in sepsis.

Moreover, the use of mechanical ventilation in the thrombocytopaenia group was higher than that in the non-thrombocytopaenia group (68.7% vs 62.9%), although no significant difference was observed. We presume that this similarity may be due to the early provision of treatment. Mechanical ventilation is a challenging technique and may create many mechanical ventilation-related lung injuries that are difficult to treat; thus, it is worthwhile to reduce the dependence on mechanical ventilation.

Two studies have suggested that thrombocytopaenia does not cause serious complications such as shock.[5,6] However, some studies have suggested that thrombocytopaenia causes a higher incidence of shock. The current meta-analysis demonstrated that the incidence of overall complications was significantly lower in the non-thrombocytopaenia group. Our review showed that shock occurred in sepsis in the thrombocytopaenia group but occurred less frequently in the non-thrombocytopaenia group. Furthermore, bleeding was rarer in the non-thrombocytopaenia group (0.823% vs 5.79%). For other complications such as ARDS and AKI, our findings are similar to those of the previous studies.[6–9] Our review showed that ARDS (37.4% vs 31.9%) and AKI (44.1% vs 26.7%) occurred more frequently in sepsis in the thrombocytopaenia group.

We conducted a sensitivity analysis by excluding some studies, and the result of the original analysis did not change. Furthermore, our meta-analysis included a recently published well-designed trial by Thiery-antier *et al.*[10] Our meta-analysis showed that thrombocytopaenia increased the incidence of overall complications, and this result did not change by restricting the meta-analysis to high-quality studies. In addition, our meta-analysis showed that the use of mechanical ventilation in the thrombocytopaenia group was significantly decreased. A strength of this study is the large sample size analysed. Our study is the only comprehensive meta-analysis of the outcomes of sepsis with or without thrombocytopaenia in over 6000 adults.

In conclusion, thrombocytopaenia is associated with poor prognosis and increases the complication rate in sepsis. This suggests that platelet count in septic patients could be used to indicate the development of one or more of these prognostic values in septic patients. These trends provide a reference for further clinical treatment.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors agree to publish.

Conflict of interests

The authors declare that they have no competing interests.

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Authors' contributions

All the authors fulfill all three authorship criteria: conception and design or analysis and interpretation of data, drafting the article and revising it critically for important intellectual content and the final approval of the version to be published. All the authors read and approved the final manuscript.

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Availability of data and material

Please contact author for data requests.

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Detection of pediatric bacterial meningitis pathogens from cerebrospinal fluid by next-generation sequencing technology

Dear Editor,

Breuer and co-workers , in this Journal, held the opinion that next generation sequencing (NGS) should be considered as a frontline diagnostic test in chronic and recurring encephalitis.[1] There is increasing evidence of a role for NGS in the work-up of central nervous system (CNS) infection. Pathogen identification is of paramount importance for bacterial meningitis. Because of the limitations of clinical laboratory testing, more than half of the CNS infection cases cannot be clearly diagnosed.[2] Although non-culture methods including multiplex PCR and latex agglutination, etc. have been used in clinical microbiology,[3] only one or several specific pathogens could be targeted by these technologies, let alone rare pathogens. Thus, rapid and accurate diagnosis of causative agents in CNS infection remains challenging. Unbiased NGS could facilitate identification of all the potential pathogens in a single assay theoretically.[4] However, the majority of reports of CNS infection are case reports and few studies applied NGS for pathogen detection of bacterial meningitis patients, especially in pediatric populations. In this study, we would like to use the NGS technology to detect pathogens directly from the CSF samples of children with bacterial meningitis and evaluate the feasibility and significance of the NGS technique on the pathogenic identification of bacterial meningitis.

This retrospective observational study was conducted between 23rd October, 2014 and 31st December, 2016. This study was approved by the Ethics Committee of Beijing Children's Hospital Affiliated to Capital Medical University (No. 2017-74). Patients with bacterial meningitis age between 29 days and 18 years from Department of Infectious Diseases, Beijing Children's Hospital were included. The inclusion criteria of bacterial diagnosis were according to the World Health Organization case definition.[5] The exclu-

Abbreviation: NGS, next generation sequencing (NGS); CNS, central nervous system (CNS); S. pneumoniae, Streptococcus pneumoniae; S. Agalactiae, Streptococcus agalactiae; Spn, Streptococcus pneumoniae; GBS, group B Streptococcus; SA, Staphylococcus aureus; Eco, Escherichia coli; Li, Listeria monocytogenes.

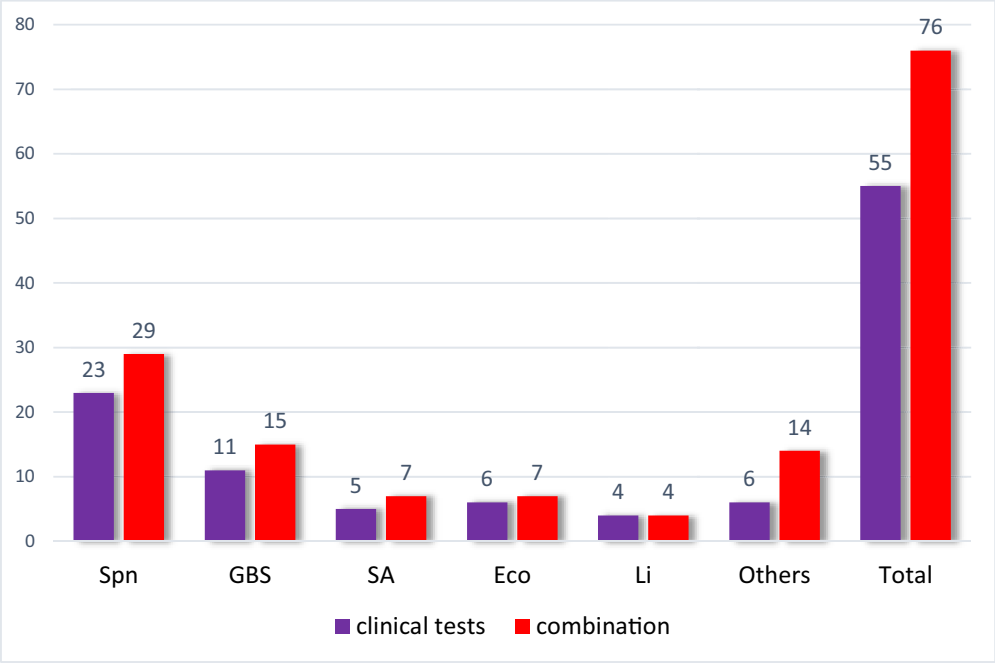

Fig. 1. Number of different pathogens detected by clinical tests and combination with NGS. *Note:* Spn, *Streptococcus pneumoniae*; GBS, *group B Streptococcus*; SA, *Staphylococcus aureus*; Eco, *Escherichia coli*; Li, *Listeria monocytogenes;* others includes *Enterococcus faecalis, Enterococcus faecalis, Streptococcus pyogenes, Streptococcus mitis, Streptococcus bovis, Haemophilus influenza, Streptococcus bovis, Eikenellacorrodens, Pseudomonas aeruginosa* and *Staphylococcus pasteuri, Cytomegalovirus and Taeniasaginataasiatica.*

sion criteria were patients with disagreement to collecting CSF, CSF volume < 1 ml and bloody CSF. Records of all patients including demographic data, clinical features and laboratory findings were obtained. CSF samples were collected and sent to Beijing Genomics Institute for pathogen detection using NGS technique.

A total of 99 cases were finally included in our study. Among them, 68 (68.7%) cases were male. The median age of onset was 5.7 months. 55 cases (55.6%) had positive clinical pathogen detections. Among them, 33 (33.3%) cases were positive from the CSF culture, 32 (32.3%) cases were positive from the blood culture, and 13 (13.1%) cases of them were positive both from CSF and blood culture. 21 (21.2%) cases had positive results of Alere BinaxNow® *Streptococcus pneumoniae* Antigen test. The main pathogens identified in this study was *Streptococcus pneumoniae (S. pneumoniae)* (n = 23, 41.8%). A total of 10 kinds of pathogenic microorganisms were determined by NGS. There were six kinds of Gram-positive bacteria, two kinds of Gram-negative bacteria, one kind of virus and one kind of parasite among them. The coverage, depth and unique reads of different spices were between 0.0046% and 68%, 1-2, 2-67550, respectively (Supplemental Table S1). The accordance rate of NGS and conventional microbiologic methods was 48.5% (n = 48) with 17 samples positive and 31 samples negative by both methods; 13 samples tested negative by conventional methods were tested positive by NGS. NGS showed higher sensitivity and increased the positive rate of pathogen detection by 13.1% (the positive rate of pathogen detection increased from 55.6% to 68.7%). A total of 29 *S. pneumoniae* were detected by combination methods, while six of them were independently detected by NGS; a total of 15 *Streptococcus agalactiae (S. agalactiae)* were detected by combination methods, while four of them were independently detected by NGS. Overall, *S. pneumoniae* (n = 29, 39.7%), *S. agalactiae* (n = 15, 20.5%), *Staphylococcus aureus* (n = 7, 9.6%), and *Escherichia coli* (n = 7, 9.6%) were the most frequently detected pathogens in this study (Fig. 1). The NGS-positive group comprised 41 (41.4%) patients while the NGS-negative group comprised 58 cases. Comparing the two groups, patients in the NGS-positive group had generally shorter days in terms of duration from onset to the sample collection (median days 15 vs. 31 days, P = 0) and shorter onset days than those in the pathogen-negative group (median days 6 vs. 25 days, P = 0.005). With regard to blood inflammatory indices, patients in the NGS-positive group were frequently found to have higher neutrophils (66.7% vs. 54.7%, P = 0.002) than those in the NGS-negative group (Table 1). The main pathogens identified in this study were *S. pneumoniae* which was consistent with our prior data.[6] NGS increased the positive rate of pathogen detection by 13.1% in our study which indicated NGS could improve the overall positive rate of pathogen detection in bacterial meningitis patients. This consistent with the studies of NGS in patients with other infectious diseases,[7] such as sepsis[8] and pneumonia[9] which indicates that an NGS-based approach has great potential to detect causative pathogens of infectious diseases.

We compared NGS-positive and NGS-negative groups, and analyzed factors that could affect NGS results. Patients in the NGS-positive group had generally shorter days in terms of duration from onset to the sample collection than those in the pathogen-negative group. In terms of time between two groups, we found that 32 (94.12%) samples were less than 42 days (data did not show) in the NGS-positive group. It indicated that the earlier detected the more positive possibility. In addition, it is noting that there was no significant difference in the simultaneously CSF white blood cell count, protein and glucose levels between two groups. These altogether suggests that the original severity of inflammation, not the inflammation status while the simultaneously NGS detects might determine the NGS results.

To our knowledge, the present study is the largest case series of using NGS on the pathogen identification of bacterial meningitis patients around world. Our study demonstrated the diagnostic ability of NGS in determines etiology of bacterial meningitis and the factors associated with the NGS results. Although there are many challenges, especially on the interpre-

Table 1
Comparison of NGS-positive and NGS-negative groups.

Items		NGS-positive[b] ($n=41$)	NGS-negative ($n=58$)	*P*
Age (days)[a]		230(69.5–425)	116.5(65.5–392.5)	0.379
Duration from onset to the sample collection (days)[a]		15(8.5–28)	31(17–45)	0
Onset (days)[a]		6(4–21)	25(4–41)	0.005
Diagnosis duration (days)[a]		4(2–8)	3(1–6.75)	0.66
Peripheral blood	WBC(× 10^9/l)	17.3 ± 11.9	13.5 ± 7.3	0.074
	Neutrophils (%)	66.7 ± 15.7	54.7 ± 20.2	0.002
	Hemoglobin (g/l)	104.0 ± 19.9	103.8 ± 17.2	0.943
	C-reactive protein (mg/l)[a]	29 (11.0–101.1)	27.7(8–108.5)	0.393
1st CSF	WBC (× 10^6/l)[a]	910(336–2825)	403(98–2040)	0.125
	Multinuclear cells (%)[a]	70(38.75–85)	60(22–75)	0.165
	Monocyte (%)[a]	24.3(15–55.5)	38.5(23.0–75.0)	0.099
	Protein (mg/l)[a]	1278(837–2234)	1340(596–1982)	0.485
	Glucose (mmol/l)[a]	1.47(0.87–2.74)	2.48(1.02–2.48)	0.12
Simultaneously CSF[c]	WBC (× 10^6/l)	10(2–17)	11(2–37)	0.432
	Multinuclear cells (%)[a]	10(4–16)	12(5–20)	0.723
	Monocyte (%)	20(12–28)	30(15–54)	0.051
	Protein (mg/l)[a]	557(296–1149)	594(367–1079)	0.85
	Glucose (mmol/l)[a]	2.85(2.43–3.46)	2.90(2.46–3.66)	0.869

NGS, next generation sequencing; WBC, white blood cell; CRP, C-reactive protein; CSF, cerebrospinal fluid.
[a] Results are reported as the median (interquartile range), or as the percentage.
[b] NGS-positive group refers to the cases who had positive bacterial NGS results.
[c] Simultaneously CSF refers to the CSF results by clinical testing at which time CSF is detected by NGS.

tation of NGS report. As a new technology of detection, NGS could be a promising alternative diagnostic powerful tool for pathogen detection allowing doctors to detect organisms in critically ill patients suffering from bacterial meningitis quickly and precisely.

Author contributions

All of the authors had access to the full dataset (including the statistical reports and tables) and take responsibility for the integrity of the data and the accuracy of the data analysis. GLY, LYJ, YYH and LG conceived and designed the study. GLY, LLL, ZY, FWY, ZL, HB, HHL, CHY, CTM, GX were involved in case and sample collection, analysis, and interpretation of the data. GLY, LYJ, WHL, ZJL participated in the laboratory analysis of CSF. GLY wrote the first draft of the paper. GLY, YYH, and LG reviewed and approved the final report.

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Conflict of interest

The authors declare that they have no competing interests.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2018.12.001.

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Evaluation of next-generation sequencing for the pathogenic diagnosis of children brain abscesses

Dear Editor,

Guo et al.,[1] in this Journal, held the view that metagenomic next-generation sequencing (mNGS) may hold the comprehensive diagnostic ability in identifying different sources of abscess pathogens and indicated that this technology provides a powerful ability for the rapid etiology diagnosis of patients with abscess. We completely agree with the opinion that mNGS was suggested to potentially replace traditional microbiological methodology because of its comprehensiveness and speed. However, clinical experience with application of the test is relatively limited, especially for samples of brain abscesses.

Internal organ abscesses are illustrative of the shortcomings of bacterial culture. They often contain anaerobic or fastidious bacteria vulnerable to sample collection procedures and transportation, and they can also contain atypical or slow-growing organisms that will not necessarily form colonies on routine media or during a standard incubation time. NGS has the ability to identify both common and rare pathogens without any prior suspicious needed, and is able to offer a new platform for quantification of all detected microorganisms.[2] Here we present a group of children brain abscess cases, where NGS was used for pathogenic confirmation.

Case 1, a 2-month-old baby was sent to local emergency room (ER) because of continuous fever with spit. His brain Magnetic Resonance Imaging (MRI) revealed multiple round abnormal signal in the left frontal lobe (Fig. 1(A)). His blood routine test showed a white blood cell count of 24.44×10^9/L, with 40.4% granulocytes, and a markedly increased C-reactive protein (CRP) level (92 mg/L). Intravenous ceftriaxone, vancomycin and mannitol were prescribed but the baby's fever did not relieve and abdominal distension appeared.

Case 2, a 5-year-old boy, who had a history of congenital heart disease, was sent to local ER for intermittent fever with headache and vomit. His brain CT revealed the massive low-density shadow sorrounded by large areas of edema in the left temporal-occipital-parietal lobe (Fig. 1(B)). His blood routine test showed a white blood cell count of 16.43×10^9/L, with 47.6% granulocytes. His cerebrospinal fluid (CSF) examination revealed increased leucocyte count (190×10^6 cells/L) with normal glucose and slightly elevated protein level (973 mg/L). Ceftriaxone and mannitol were prescribed and symptoms relieved transiently. Over the next 3 days, fever with severe headache and fatigue recurred, and he was admitted to our hospital.

Case 3, a 10-year-old boy went to ER because of continuous fever, speech disorder and convulsion. Cranial MRI indicated abnormal lesions in left frontal parietal junction (Fig. 1(C)). His CSF examinations revealed increased leucocyte count (31×10^6 cells/L) with normal glucose and protein level. Empirical antibiotic treatment including intravenous cefepime, ganciclovir were administered but symptoms were not relieved.

Case 4, a 13-year-old previously healthy boy went to local hospital for intermittent fever with headache. His temperature peak was 39.1 °C with severe headache and personality change. His blood routine test showed increased white blood cell count (13.94×10^9/L), with 87.5% granulocytes. CRP was 68 mg/L. His cranial MRI showed the circular abnormal signal in the left frontal lobe (Fig. 1(D)). Ceftriaxone and mannitol were prescribed, but failed to relieve the symptoms.

All 4 patients were then transferred to our hospital, NGS and culture of abscess samples were performed. Pathogenic information of brain abscess samples from 4 patients are summarized in Fig. 1 and Table 1. All the four patients were treated with antibiotics before admitted to our hospital, however, NGS detected *Bacteroides fragilis* in patient 1, *Streptococcus intermedius* and *Streptococcus constellatus* in patient 2, *Streptococcus intermedius* in patient 3, and *Prevotella oralis, Fusobacterium nucleatum* and *Streptococcus intermedius* in patient 4, consistent with the simultaneous abscess samples culture results in patient 1 and patient 2. Our study showed being applied to brain abscess samples, mNGS could yield a higher sensitivity for pathogen identification and is less affected by prior antibiotic exposure.

For patient 1 and patient 2, whose culture of abscess samples were positive, the sequencing reads (5165, 5566) of *Bacteroides fragilis* and *Streptococcus intermedius*, respectively, were higher than those of other two culture-negative patients. For patient 2, although he had a long present history about thirty days when admitted to our hospital, his reads of NGS was still high, which may

Abbreviations: NGS, next-generation sequencing; CNS, central nervous system; CRP, C-reactive protein; MRI, magnetic resonance imaging; ER, emergency room; CSF, cerebrospinal fluid.

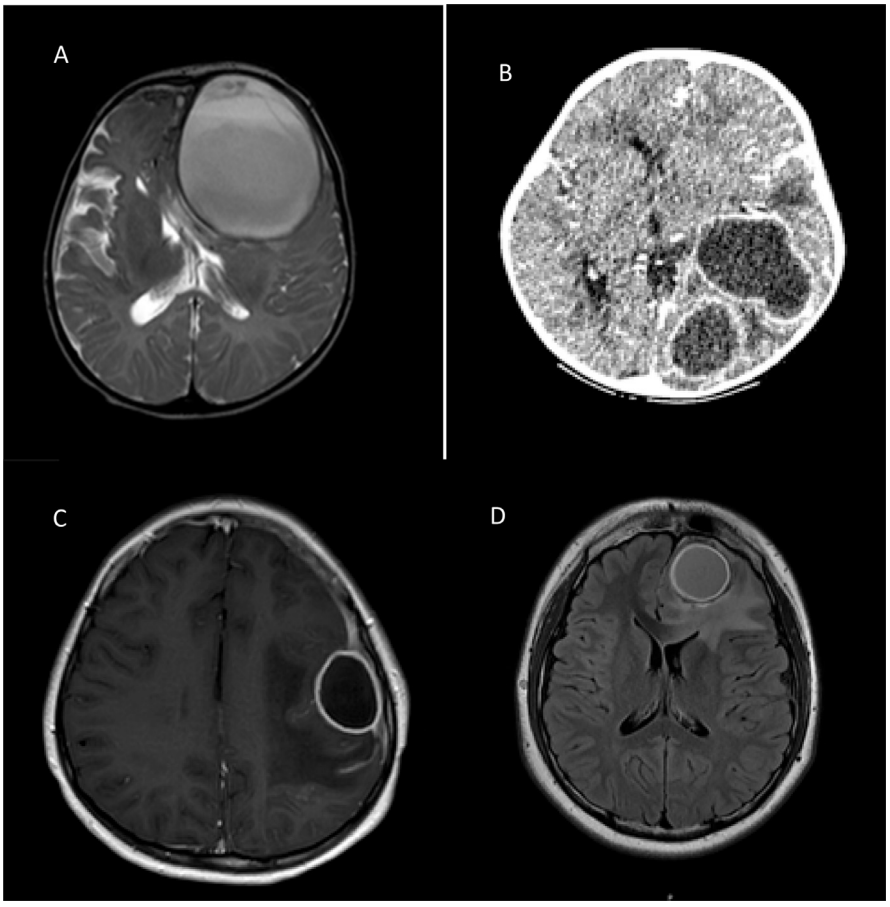

Fig. 1. Cranial MRI/CT findings of 4 patients.
An axial T2-weighted image of cranial MRI revealed the circular abnormal signal in the left frontal lobe with liquid plane in the cyst and compressive displacement of midline structure, bilateral frontal lobes and lateral ventricle (Panel A).
An cranial enhanced CT revealed the cystic space-occupying lesions in the left cerebral hemisphere with wall enhancement, peripheral brain edema and right shift of midline structure (Panel B, the patient with metal implants after cardiac surgery should not undergo MR examination).
An axial T1 cranial MRI revealed the circular enhanced signal in the left fronto-parietal junction area and the patchy long T1 signal in the left fronto-parietal white matter. The left fronto-parietal temporal meninges were thickened and enhanced (Panel C).
An axial T2 flare cranial enhanced MRI showed uneven thickness of the left frontal lobe lesion wall, circular enhancement, mild enhancement of the surrounding brain parenchyma, lesions involving the hypothalamus, and perifocal edema. Left frontal meningeal thickening and enhancement (Panel D).

Table 1
Pathogenic and antimicrobial treatment information of four patients with brain abscess.

No.	Age	NGS results		Culture results		Antimicrobial treatment	
		Bacteria (reads)	Virus/Fungi/ Parasite	Common bacteria culture	Anaerobic bacteria culture	Before NGS	After NGS
1	2-month-old	*Bacteroides fragilis (5165)* *Bacteroides xylanisolvens (131)* *Alistipes shahii (9)* *Alistipes finegoldii (3)* *Odoribacter planchnicus (18)* *Porphyromonas gingivalis (4)*	None	None	*Bacteroides fragilis*	Meropenem and Vacomycin	Meropenem
2	5-year-old	*Streptococcus intermedius (5566)* *Streptococcus constellatus (143)* *Fusobacterium 4 (23)* *Fusobacterium 3 (3)* *Campylobacter curvus (8)* *Filifactor alocis (8)*	None	*Streptococcus intermedius*	*Streptococcus intermedius*	Meropenem and Vacomycin	Vancomycin
3	10-year-old	*Streptococcus intermedius (139)* *Streptococcus constellatus (3)*	None	None	None	Ceftriaxone and vancomycin and metronidazole	Ceftriaxone and metronidazole
4	13-year-old	*Prevotella oris (32)* *Fusobacterium nucleatum (3)* *Streptococcus intermedius (3)*	None	None	None	Ceftriaxone and vancomycin and metronidazole	Ceftriaxone and vancomycin and metronidazole

demonstrate the good diagnostic value of NGS applied in samples of abscess.

Any delay precision antimicrobial treatment may lead to poor outcome of brain abscesses, unnecessary broad-spectrum antibiotic usage and induce antibiotic resistance. With the NGS detection, all the four patients were treated with the appropriate antibiotic treatment timely (Table 1). All the patients were completely recovered after all.

Currently, NGS is a comprehensive approach for sequence-based identification of pathogenic microbes.[3–6] However, reports on the use of NGS in the field of internal organ abscesses,[7,8] especially brain abscesses, are really rare. Kommedal et al.[9] reported fifty-two surgical samples of brain abscess were detected by massive parallel sequencing (MPS) and *Aggregatibacter aphrophilus, Fusobacterium nucleatum,* and *Streptococcus intermedius* or combinations of them were found in all spontaneous polymicrobial abscesses. This strengthen was once again confirmed by our study. Hence, for patient 4, although the reads of NGS were low (Table 1), we still believed that *Prevotella oralis, Fusobacterium nucleatum* and *Streptocuccus intermedius* were causative pathogens. Furthermore, the abscess sample of patient 4 was got at his convalescence, so the low reads of NGS could be explained by Ai's[2] and Guo's[1] opinion that NGS may have the potential to be an encouraging tool by reflecting disease states through its semi-quantitive surveillance of pathogen loads.

But, in study of Kommedal et al., bacterial 16S rRNA genes were amplified directly from the specimens and sequenced using Ion Torrent technology, which is different from the new generation of high-throughput and low-cost sequencing technology what we discussed in our study.

In this study, we originally performed metagenome analysis by NGS on brain abscess from four patients, which is more rapid and more comprehensive than conventional NGS. Our study demonstrated the rapid and comprehensive diagnostic ability of NGS in identifying unknown etiology from samples of brain abscess, and displayed the possible potential that NGS is about to provide the diagnostic tools that can characterize even the most complex microbial communities during brain abscesses and is less affected by prior antibiotic exposure.

Declaration

We confirm that each individual named as an author meets the journal's criteria for authorship and neither the entire paper nor any part of its content has been published or accepted elsewhere. It is not being submitted to any other journal.

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Conflict of interest

No potential conflicts of interest.

Acknowledgments

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