Diagnostic and prognostic utilities of humoral fibulin-3 in malignant pleural mesothelioma: Evidence from a meta-analysis

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ABSTRACT

Fibulin-3 has emerged as a promising novel biomarker in conforming or monitoring malignant pleural mesothelioma (MPM). This study sought to evaluate the diagnostic and prognostic efficacies of humoral fibulin-3 for MPM. Seven eligible publications comprising 468 MPM cases for diagnosis, and 138 for prognosis were identified. Results manifested that humoral fibulin-3 sustained a pooled sensitivity of 0.62 (95% CI: 0.45–0.77) and specificity of 0.82 (95% CI: 0.73–0.89) in discriminating MPM patients from cancer-free individuals, corresponding to an AUC (area under the curve) of 0.81. For the survival analysis, fibulin-3 expression was not markedly associated with overall survival (OS) time of the MPM patients [HR (hazard ratio): 1.84, 95% CI: 0.75–4.56, \( P = 0.185 \)]. In the subgroup analyses stratified by test matrix and ethnicity, data revealed that serum-based fibulin-3 examination achieved superior accuracy than plasma-based analysis (sensitivity: 0.77 versus 0.54; specificity: 0.85 versus 0.77; AUC: 0.92 versus 0.69); additionally, testing of fibulin-3 in Europeans retained higher efficacy than those in Americans and Australians. Taken together, fibulin-3 confers a relatively high diagnostic efficacy and is acceptable to be an auxiliary biomarker to aid in MPM identification.

INTRODUCTION

Malignant pleural mesothelioma (MPM) is a universally fatal neoplasm with high lethality features of easy metastasis and poor clinical outcomes [1–4]. The etiology of MPM is strongly linked to previous exposure to asbestos, although other aetiopathogenetic factors could not be eliminated [5, 6]. Despite the new advantages in therapeutics, the median survival time of patients with MPM remains dissatisfying, which only ranged from 6–18 months following diagnosis [3, 6]. Timely diagnosis of MPM is limited by the long latency stage in clinic, on this point, developing reliable diagnostic and/or prognostic markers for MPM will significantly benefit and enhance the clinical care.

Human fibulin-3, encoded by the epidermal growth factor-containing fibulin-like extracellular matrix protein-1 (EFEMP-1) gene, is identified as a secreted glycoprotein and plays an essential role in regulation of cell proliferation and migration [7, 8]. Recent findings have documented the significantly altered expression status of humoral fibulin-3 in mesothelioma, thereby highlighting its promising application as a novel biomarker for MPM diagnosis and prognosis [9–18]. Nevertheless, single study often presented with inaccurate and insufficient information due to restrictions from limited sample size and research programs. For instance, a study from Pass et al held that fibulin-3 was adequately sensitive and specific in confirming MPM, with a diagnostic sensitivity and specificity up to 90% [12]. However, some research reported that fibulin-3 testing only yielded a diagnostic sensitivity from 12.7% to 59%, and therefore questioned the application of fibulin-3 as a marker for MPM [10, 15]. Upon the above arguments, we conducted a comprehensive meta-analysis and aimed to evaluate the clinical utility, including the diagnostic and prognostic capabilities of humoral fibulin-3 for MPM.
RESULTS

Study features and article quality

According to the predefined criteria, 83 citations were obtained from the online PubMed database, yet 4 records were obtained through a search of article references. As shown in Figure 1, 79 records were excluded either due to the status of reviews, basic research articles or the contents were unrelated to fibulin-3 diagnosis or prognosis. Eight eligible studies then received full text evaluation, and in one of them, the ROC curve analysis compared the status of fibulin-3 in monitoring chemotherapy response of MPM, data from which were eventually eliminated [19]. Last, 6 studies for diagnosis [9, 10, 12, 14, 15, 17], and 3 for prognosis [10, 15, 16], were enrolled for the meta-analysis.

The main characteristics of each study are summarized in Table 1. A total of 468 MPM patients and 664 matched controls for the diagnostic analysis, and 138 MPM cases for prognostic category were obtained. All patients with MPM were prior to treatment and the final diagnosis all relied on the histopathological examinations. The control sources comprised healthy participants, asbestos-exposed individuals and non-MPM related effusions. The specimen type involved plasma, serum and effusion, and abundance of fibulin-3 was determined by enzyme linked immunosorbent assay (ELISA) in humoral. Study population consisted of Australians, Americans and Europeans. In the survival analysis, the primary endpoint of overall survival (OS) was extracted and the follow-up time was from 12 to 20 months.

The included cohorts were evaluated by the QUADAS II and Newcastle-Ottawa Scale (NOS) checklists [20, 21]. Figure 2 indicated the proportions of diagnostic studies with low, high, or unclear concerns regarding risk of bias and applicability by QUADAS II list. Scores of the prognostic studies judged by the NOS checklist were listed in Supplementary Table 1.

Heterogeneity

Exploring of study heterogeneity was enabled by the testing of threshold and non-threshold effects among
studies [22]. As exemplified in Table 2, Spearman’s correlation coefficient of the overall pooled diagnostic analysis after adjustment was estimated to be 0.254, with a \(P\) value of 0.362, hinting that no significant heterogeneity was generated due to threshold effect. On the other hand, Cochran’s-Q and \(I^2\) tests were performed to analyze heterogeneity from non-threshold effect, wherein, heterogeneity appeared in the overall pooled analysis after adjustment (Cochran’s-Q = 80.06, \(P = 0.0000\), \(I^2 = 82.5\%\)), as well as in the stratified analyses (Table 2). For the prognostic analysis, the \(I^2\) was estimated to be 87.3\%, also revealing a significant heterogeneity in studies. As a result, a random effect model was finally employed for the combined analyses.

**Table 1: Main features of the included studies for humoral fibulin-3 in identification of MPM**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Area</th>
<th>Case vs. Control type</th>
<th>Asbestos exposure (%) or years</th>
<th>Patient vs. Control size</th>
<th>Test method</th>
<th>Sample type</th>
<th>Cut-off value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaya et al. [9]</td>
<td>2015</td>
<td>Turkey</td>
<td>MPM vs. Normal</td>
<td>83.7%</td>
<td>43 vs.40</td>
<td>ELISA</td>
<td>Serum</td>
<td>30.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MPM vs. Non-MPM</td>
<td>83.7%</td>
<td>43 vs.40</td>
<td>ELISA</td>
<td>Plasma</td>
<td>29.0</td>
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<tr>
<td>Creaney et al. [10]</td>
<td>2014</td>
<td>Australia</td>
<td>MPM vs. Non-MPM</td>
<td>Unclear</td>
<td>82 vs.120</td>
<td>ELISA</td>
<td>Plasma</td>
<td>52.0</td>
</tr>
<tr>
<td>Pass et al. [12]</td>
<td>2014</td>
<td>America</td>
<td>MPM vs. Non-MPM</td>
<td>52.3 or 95.8%</td>
<td>92 vs.290</td>
<td>ELISA</td>
<td>Plasma</td>
<td>52.8</td>
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<td></td>
<td></td>
<td></td>
<td>MPM vs. Non-MPM</td>
<td>52.3 or 95.8%</td>
<td>92 vs.136</td>
<td>ELISA</td>
<td>Plasma</td>
<td>67.1</td>
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<tr>
<td>Demir et al. [17]</td>
<td>2015</td>
<td>Turkey</td>
<td>MPM vs. Normal</td>
<td>42.9 ± 19.1 years</td>
<td>42 vs.41</td>
<td>ELISA</td>
<td>Sera</td>
<td>51.4</td>
</tr>
<tr>
<td>Napolitano et al. [14]</td>
<td>2016</td>
<td>America</td>
<td>MPM vs. Normal</td>
<td>Unclear</td>
<td>22 vs.20</td>
<td>ELISA</td>
<td>Serum</td>
<td>Unclear</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>MPM vs. Non-MPM</td>
<td>Unclear</td>
<td>22 vs.25</td>
<td>ELISA</td>
<td>Serum</td>
<td>Unclear</td>
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<tr>
<td>Kirschner et al. [15]</td>
<td>2015</td>
<td>Australia</td>
<td>MPM vs. Non-MPM</td>
<td>Unclear</td>
<td>37 vs.32</td>
<td>ELISA</td>
<td>Plasma</td>
<td>29.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>MPM vs. Non-MPM</td>
<td>Unclear</td>
<td>47 vs.24</td>
<td>ELISA</td>
<td>Plasma</td>
<td>29.0</td>
</tr>
</tbody>
</table>

MPM: malignant pleural mesothelioma; ELISA: enzyme linked immunosorbent assay, vs: versus.

Influence analysis and diagnostic accuracy

As the existence of significant heterogeneity among studies which may further compromise the overall accuracy of pooled analyses, we conducted influence analysis to deeply trace the outlier studies. Data showed that 2 individual studies were evaluated as outliers and were finally eliminated (Supplementary Figure 1). Accordingly, the adjusted meta-analyses manifested that humoral fibulin-3 yielded a pooled sensitivity of 0.62 (95% CI: 0.45–0.77), specificity of 0.82 (95% CI: 0.73–0.89) and AUC of 0.81 in differentiating MPM patients from cancer-free participants (Figure 3A–3C). Meanwhile, the pooled PLR (positive likelihood ratio), NLR (negative likelihood ratio), and DOR (diagnostic odds ratio) were estimated to be 3.44 (95% CI: 2.24–5.29), 0.46 (95% CI: 0.31–0.69), and 7.44 (95% CI: 3.63–15.25), respectively, corresponding to a diagnostic score of 2.007 (95% CI: 1.290–2.724) (Table 3).

Fagan’s plot assay displayed apparent improvements of post-test probabilities in the pooled analysis, with a post-test probability of positive result of 46% and negative result of 10% (Figure 3D).

**Subgroup analyses and meta-regression**

Subgroup analyses were stratified in terms of test matrix and study ethnicity. The analysis of fibulin-3 efficacy on sample type revealed that serum-based assay harbored higher diagnostic accuracy than plasma-based analysis (sensitivity: 0.77 vs. 0.54; specificity: 0.85 vs. 0.77; DOR: 29.11 vs. 3.55; AUC: 0.92 vs. 0.69). Additionally, analysis based on ethnicity also showed that testing of fibulin-3 in Europeans sustained better efficacy than those in Americans and Australians (Table 3).

Sources of heterogeneity were deeply explored by the univariate meta-regression test with the predefined variations included test matrix, ethnicity, cut-off value, QUADAS score, patient and control sizes, and so forth [22]. As exemplified in Supplementary Table 2, the test matrix (\(P = 0.0052\)) and control size (\(P = 0.0207\)) were more likely to be the main causes of heterogeneities among studies.

**Prognosis**

Association between fibulin-3 expression and overall survival (OS) time was investigated in 3 studies...
As shown in Figure 4, fibulin-3 level was not significantly associated with OS time in MPM patients (HR: 1.84, 95% CI: 0.75–4.56, \( P = 0.185 \)).

Publication bias

The \( P \) values of Deeks’ funnel plot asymmetry test and Egger’s tests were estimated to be 0.749 and 0.947, respectively, while Egger’s test also yielded a corrected \( z \) value of 0.734 (indicating \( P > 0.05 \)), suggesting that no significant publication bias existed in the meta-analyzed studies (Figure 5 and Supplementary Figure 2).

DISCUSSION

Malignant pleural mesothelioma (MPM) is one kind of asbestos-related and highly aggressive neoplasm evolves with a median life expectancy of 6–18 months...
### Table 3: The pooled analyses of diagnostic efficacy of fibulin-3 in confirming MPM

<table>
<thead>
<tr>
<th>Analysis</th>
<th>AUC</th>
<th>Pooled sensitivity (95% CI)</th>
<th>Pooled specificity (95% CI)</th>
<th>Pooled DOR (95% CI)</th>
<th>Pooled PLR (95% CI)</th>
<th>Pooled NLR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (adjusted)</td>
<td>0.81</td>
<td>0.62 (0.45–0.77)</td>
<td>0.82 (0.73–0.89)</td>
<td>7.44 (3.63–15.25)</td>
<td>3.44 (2.24–5.28)</td>
<td>0.46 (0.31–0.70)</td>
</tr>
<tr>
<td>Overall (unadjusted)</td>
<td>0.74</td>
<td>0.61 (0.46–0.74)</td>
<td>0.76 (0.61–0.87)</td>
<td>5.03 (2.12–11.95)</td>
<td>2.58 (1.47–4.51)</td>
<td>0.51 (0.35–0.75)</td>
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<tr>
<td>Semen type</td>
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<tr>
<td>Plasma</td>
<td>0.69</td>
<td>0.54 (0.50–0.58)</td>
<td>0.77 (0.74–0.80)</td>
<td>3.55 (2.01–6.27)</td>
<td>2.03 (1.39–2.97)</td>
<td>0.68 (0.51–0.89)</td>
</tr>
<tr>
<td>Serum</td>
<td>0.92</td>
<td>0.77 (0.71–0.83)</td>
<td>0.85 (0.79–0.90)</td>
<td>29.11 (9.66–87.74)</td>
<td>5.46 (2.84–10.51)</td>
<td>0.20 (0.07–0.56)</td>
</tr>
<tr>
<td>Ethnicity</td>
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</tr>
<tr>
<td>Australian</td>
<td>0.59</td>
<td>0.40 (0.35–0.46)</td>
<td>0.78 (0.73–0.82)</td>
<td>2.45 (1.44–4.17)</td>
<td>1.85 (1.13–3.04)</td>
<td>0.83 (0.77–0.90)</td>
</tr>
<tr>
<td>American</td>
<td>0.80</td>
<td>0.71 (0.66–0.75)</td>
<td>0.76 (0.73–0.79)</td>
<td>7.44 (3.55–15.60)</td>
<td>2.61 (1.70–4.02)</td>
<td>0.39 (0.26–0.59)</td>
</tr>
<tr>
<td>European</td>
<td>0.91</td>
<td>0.75 (0.68–0.81)</td>
<td>0.84 (0.78–0.90)</td>
<td>25.46 (5.51–117.71)</td>
<td>5.23 (2.10–13.06)</td>
<td>0.22 (0.06–0.83)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the curve.

**Figure 3:** Forest plots of (A) pooled sensitivity, (B) specificity, (C) SROC curve and (D) post-test probability for humoral fibulin-3 in confirming MPM.
Patients suffering from MPM often present with non-specific symptoms and therefore result in delayed diagnosis and late-stage disease [1–4]. In 2002, a remarkable research reported that plasma fibulin-3 allowed for the discrimination of MPM patients from cancer-free individuals with high and promising diagnostic efficacy, and therefore strongly recommended its clinical application as a robust indicator in confirming MPM [12]. Since this report, an increasing number of investigations have focused on the diagnostic and prognostic utilities of humoral fibulin-3 in identifying or monitoring MPM [9–18]. Notwithstanding, to our knowledge and available literature, the published data of humoral fibulin-3 testing revealed a high volatility among studies and as yet remains a problem of controversy. We therefore conducted this meta-analysis and made a comprehensive evaluation of the clinic utility of humoral fibulin-3 for MPM diagnosis and prognosis.

Results from our analysis showed that humoral fibulin-3 harbored a pooled sensitivity of 0.62 at a specificity of 0.82 after the outlier-adjustment. The SROC curve presents a comprehensive evaluation of a diagnostic test performance. In our study, the SROC curve analysis displayed an AUC value of 0.81 for humoral fibulin-3 testing, revealing an overall moderate efficacy to aid in MPM diagnosis. Moreover, the diagnostic odds ratio (DOR), was evaluated to be 7.44, showing a relatively high discriminatory performance of fibulin-3 in confirming...

**Figure 4:** Forest plot of pooled HR for humoral fibulin-3 expression and overall survival (OS) time in MPM patients.

**Figure 5:** Publication bias assessed by (A) Deeks’ funnel plot asymmetry test and (B) Bgger’s funnel plot.
In conclusion, the current meta-analysis suggests that humoral fibulin-3 is acceptable to be a diagnostic biomarker for MPM. Nevertheless, our study still yielded some limitations, such as the population bias, complicated control sources as well as the small case numbers in the stratified analyses. Further studies are still warranted to more comprehensively investigate the prognostic role of humoral fibulin-3 in MPM.

MATERIALS AND METHODS

Search strategy and study selection

This meta-analysis was conducted in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) [28]. Two reviewers independently searched the online PubMed database until June 30th, 2016. The keywords for search were termed as “fibulin-3”, “mesothelioma/pleural mesothelioma/malignant pleural mesothelioma”, “sensitivity/specificity/diagnosis/accuracy/ROC” and “prognosis/survival/HR/hazard ratio”. We also manually searched the article references for study identification.

The inclusion criteria were: (1) studies addressed the diagnostic performance of fibulin-3 for MPM identification; (2) studies with sufficient data to establish the 2 × 2 table; (3) studies give a clear definition of the control types; and (4) studies give a calculated HR with 95%CI. The exclusion criteria were: (1) studies failed to clearly definite the control sources; (2) data from studies were insufficient to generate the 2 × 2 table; (3) studies with insufficient data for extracting HR; and (4) non-English articles (full text), letters, duplicate reports, review articles and conference papers, etc.

Data extraction and article quality assessment

Two reviewers extracted the data with the predesigned extraction forms included the first author, year of publication, country, patient/control size, control types (healthy people or non-cancer cases), sample types (plasma, serum or other), test method, cut-off value, sensitivity and specificity, PFS (progression-free survival), and OS, etc. For the two-stage study contains both training and validation cohorts, data of each group were regarded as independent. Any disagreement was solved by group discussion.

Article quality was judged based on the Quality Assessment for Studies of Diagnostic Accuracy
(QUADAS) II and Newcastle-Ottawa Scale (NOS) checklists [20, 21].

Statistical analyses

Statistical analyses were undertaken based on the platforms of two statistical software programs: Stata 12.0 (Stata Corporation, College Station, TX, USA) and Meta-disc 1.4 (XI Cochrane Colloquium, Barcelona, Spain). For the diagnostic meta-analysis, a bivariate model allowed for the plotting of a summary receiver operating characteristic (SROC) curve as well as the pooled sensitivity, specificity, PLR, NLR and DOR. In the prognostic analysis, the HR and its 95% CI were extracted for aggregation of the survival results. Heterogeneity from the threshold effect was evaluated by the Spearman correlation coefficient, and $P < 0.05$ indicates the existence of significant heterogeneity [22]. Cochran’s-$Q$ test and $I^2$ test were applied to detect heterogeneity from non-threshold effects ($P < 0.01$ or $I^2 > 50\%$) [22]. Moreover, influence analysis and meta-regression test were employed to trace the potential sources of study heterogeneity. Publication bias was examined by Deek’s funnel plot asymmetry test (for diagnostic studies), and Bgger’s and Egger’s funnel plots (for prognostic studies).

CONFLICTS OF INTEREST

All authors declare that there are no conflicts of interest.

REFERENCES


