RESEARCH ARTICLE

Diagnostic Value of Fluorescence in Situ Hybridization Assay in Malignant Mesothelioma: A Meta-analysis

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Abstract

The diagnosis of malignant mesothelioma (MM) remains a clinical challenge and the fluorescence in situ hybridization (FISH) assay has been reported to be one promising tool. The present meta-analysis aimed to establish the overall diagnostic accuracy of FISH for diagnosing MM. After a systematic review of English language studies, the sensitivity, specificity and other measures of accuracy of FISH in the diagnosis of MM were pooled using random-effects models. Summary receiver operating characteristic curves were applied to summarize overall test performance. Nine studies met our inclusion criteria, the pooled sensitivity and specificity for FISH for diagnosing MM being 0.72 (95% CI 0.67-0.76) and 1.00 (95% CI 0.98-1.00), respectively. The positive likelihood ratio was 34.5 (95% CI 14.5-82.10), the negative likelihood ratio was 0.24 (95% CI 0.16-0.36), and the diagnostic odds ratio was 204.9 (95% CI 76.8-546.6), the area under the curve being 0.99. Our data suggest that the FISH assay is likely to be a useful diagnostic tool for confirming MM. However, considering the limited studies and patients included, further large scale studies are needed to confirm these findings.

Keywords: Fluorescence in situ hybridization - malignant mesothelioma - meta-analysis

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Introduction

Malignant mesothelioma (MM) is a high aggressive tumor originates from mesothelial surfaces with a poor prognosis and a median survival of 9 to 14 months (British Thoracic Society Standards of Care Committee, 2007). Because of the long latency period after exposure and the widespread use of asbestos fibers for many years, the incidence of MM is increasing and is expected to rise sharply worldwide in the next 20 years (Robinson and Lake, 2005). To make an early and accurate diagnosis will be of great importance to the treatment of MM.

The diagnosis of MM is mainly based on histopathologic features, together with clinical and imaging information and other data. However, a wide range of histopathologic features may present in MM and mimic other kinds of cancer. Immunohistochemistry (IHC) examination can only provide additional support for the diagnosis of MM. The combination of several markers may be useful, but the experiences of pathologists may influence the explanation of the outcomes (Allen, 2005). Imaging examinations are not that helpful for the diagnosis of MM because many patients present with effusions. Cytologic analysis is the primary diagnostic tool in most patients (Senyiğit et al., 2000; Chapman et al., 2008), while the sensitivity of cytologic examination is not enough to screen for MM patients and there is limited role of cytology in the primary diagnosis of MM (Whitaker, 2000). In addition, the cytologic distinction between MM and reactive mesothelial cells in effusions can be quite difficult (Husain et al., 2009). To find a reliable diagnostic marker in the effusion for MM is still a challenging endeavor. One recently published meta-analysis investigated the diagnostic accuracy of soluble mesothelin-ralated peptides for MM and the pooled sensitivity was only 0.64 (Luo et al., 2010), no unique marker has been shown with both high sensitivity and specificity. So it is imperative to find a novel diagnostic tool to facilitate the diagnostic accuracy. Although the molecular pathogenesis of MM has not been well understood, studies suggested that genomic alterations play a role in the pathogenesis of MM (Takeda et al., 2012). Deletion of 9p21 locus within a cluster of genes that includes CDKN2B, CDKN2A, and MTAP is the most commonly reported chromosomal alterations in MM and is readily detectable by fluorescence in situ hybridization (FISH) assay (Musti et al., 2006; Factor et al., 2009), which is a versatile technique that allows visualization of nucleic acid sequences in their native context at the single cell level (Tsuchiya, 2011). The detection of genomic alterations by FISH seems to be feasible and helpful in confirming a diagnosis of mesothelioma in cytologic and surgical specimens. Actually, the diagnostic accuracy of FISH assay for MM has been investigated in several studies, but the exact role of FISH needs to be elucidated.

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The purposes of this study were to evaluate the overall diagnostic value of FISH in MM.

Materials and Methods

The present meta-analysis was performed according to the guidelines of the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement and with methods recommended by the Cochrane Diagnostic Test Accuracy Working Group (Leeflang et al., 2008; Moher et al., 2009)

Literature search strategies

A comprehensive literature search was conducted in Medline (using PubMed as the search engine), Embase and Cochrane database until June 1, 2012. We also reviewed the reference lists of selected research papers to identify additional relevant studies. Search keywords included "mesothelioma" and "Fluorescent in Situ Hybridization". A study was included in the meta-analysis only if it could provide both sensitivity and specificity values for FISH in the diagnosis MM. Conference abstracts were excluded because of limited data present in them. Although no language restrictions were imposed initially, for the full-text review and final analysis our resources only permitted review of English articles.

Data extraction and quality assessment

Only studies provided both the sensitivity and specificity of FISH assay were included for the present meta-analysis and each study contains more than 20 specimens. The final set of articles was assessed independently by two reviewers, the reviewers were blinded to the article details and the differences between them were solved by consensus. If one study took multiple FISH probes, we choose the probe with the best diagnostic value or the combined data supplied by the authors. The following data from each publication were retrieved: (1) author; (2) publication year; (3) participant characteristics; (4) test specimens; (5) FISH probes (6) sensitivity and specificity data; (7) methodological quality, (8) study design. If no data on the above information presented in the primary studies, we marked with "NA".

To assess trial methodology, articles were reviewed independently by two authors and given a quality score by using the QUADAS (quality assessment for studies of diagnostic accuracy, an evidence based quality assessment tool to be used in systematic reviews of diagnostic accuracy studies, maximum score 14) tools (Whiting et al., 2003).

Statistical analyses

The standard methods recommended for diagnostic accuracy meta-analyses were hired in the present study (Devillé et al., 2002). For each study, we constructed 2×2 contingency tables in which all participants were classified as having positive or negative FISH results. The following indexes of test accuracy were computed for each study: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR). The diagnostic threshold identified for each study was used to plot a summary receiver operating characteristic (SROC) curve (Walter, 2002), the area under the curve (AUC) represents an analytical summary of test performance and display the trade-off between sensitivity and specificity. An AUC of 1.0 (100%) indicates perfect discriminatory ability to distinguish cases from no cases. The average sensitivity, specificity and other related indexes across studies were calculated using a random-effects model (Lijmer et al., 2002). Spearman rank correlation was performed as a test for threshold effect. Chi-square and Fisher's exact tests were used to detect statistically significant heterogeneity across studies. All analyses were performed using one statistical software programs (Meta-DiSc for Windows; XI Cochrane Colloquium, Barcelona, Spain) and all statistical tests were two-sided, and significance was set at $p<0.05$.

Results

After independent and systematic review, nine studies using FISH assay for the diagnosis of MM were included in the present meta-analysis (Illei et al., 2003; Shin et al., 2003; Chiosea et al., 2008; Onofre et al., 2008; Chung et al., 2010; Flores et al., 2010; Savic et al., 2010; Takeda et al., 2010; Monaco et al., 2011).

Quality of reporting and study characteristics

Nine studies investigated the value of FISH assay in the diagnosis of MM were available for the meta-analysis. Diagnosis of MM patients were made based on cytological or/and histopathological findings, which are reliable for the diagnosis of MM. The specimens of FISH assay included biopsy specimens, surgical resections, pleural effusion, abdominal fluid and pericardial effusion. The FISH probes in each study are different, the detailed

Table 1. Basic Information of Included Studies

Study, year	Country	Patient No.	Specimens	Reference Standard	FISH Probes
Illei et al, 2003	USA	31	PE, PAE, AF	Histology/Cytology	CDKN2A, chromosome 9
Shin et al, 2003	USA	33	PE, FNA	Histology/Cytology	Chromosome 7, 9
Chiosea et al, 2008	USA	113	Tissues	Histology	CDKN2A (p16), chromosome 9
Onofre et al, 2008	Germany	72	PE, AF	Histology/Cytology	9p21 locus probe, chromosome 9
Chung et al, 2010	Canada	65	Tissues	Histology	p16 (9p21), chromosome 9
Flores-Staino et al, 2010	Sweden	39	PE	Histology/ICC	Chromosome 3, 7, 17, 9p21
Savic et al, 2010	Switzerland	80	PE, AF	Histology/Cytology	Chromosome 3, 7, 17, 9p21
Takeda et al, 2010	Japan	65	Tissues	Histology	CDKN2A (p16), chromosome 9
Monaco et al, 2011	USA	138	Tissues,CS	Histology/Cytology	CDKN2A (p16), chromosome 9

PE, Pleural effusion; PAE, Pericardial effusion; AF, Abdominal effusion; ICC, Immunocytochemistry; CS, Cytologic specimen

Table 2. Clinical Summary of Included Studies

Study, year	Patient No.		TP	FP	FN	TN	Study Design	QUADAS
	MM	non-MM						
Illei et al,2003	13	19	12	0	1	19	Prospective	7
Shin et al,2003	17*	17	15	0	2	17	Retrospective	9
Chiosea et al, 2008	72	40	40	0	32	40	Retrospective	9
Onofre et al, 2008	33	39	30	0	3	39	Retrospective	9
Chung et al, 2010	54	11	33	0	21	11	NA	10
Flores-Staino et al, 2010	21	18	20	1	1	17	Prospective	10
Savic et al, 2010	52	28	41	0	11	28	Retrospective	11
Takeda et al, 2010	40	25	35	0	5	25	NA	10
Monaco et al, 2011	68	70	40	0	28	70	Retrospective	11

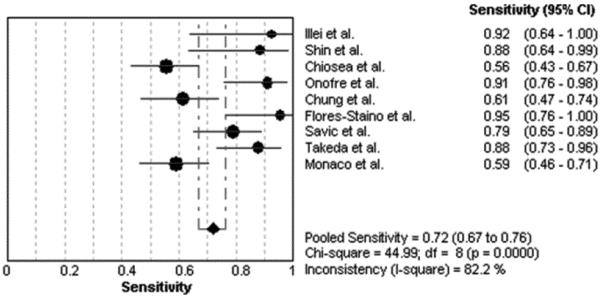

Figure 1. Forest Plots of Sensitivity for FISHA Assay for the Diagnosis of MM. The point estimates of sensitivity from each study are shown as solid circles. Error bars indicate 95% confidence intervals

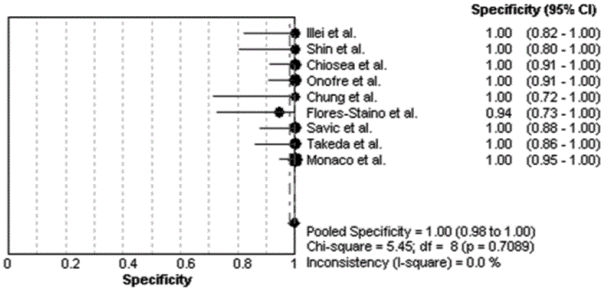

Figure 2. Forest Plots of Specificity for FISH Assay for the Diagnosis of MM. The point estimates of specificity from each study are shown as solid circles. Error bars indicate 95%

author, specimens, and FISH probes information were summarized in Table 1.

Of the nine studies of FISH in the diagnosis of MM, five had QUADAS scores ≥10. Five studies were designed as retrospective studies, only two studies were prospective studies. The clinical information of included studies was summarized in Table 2.

Diagnostic accuracy

The forest plots of sensitivity and specificity of FISH assays for the diagnosis of MM were shown in Figure 1 and Figure 2. The sensitivity ranged from 0.56 to 0.95 (pooled 0.72, 95% CI 0.67-0.76), while specificity ranged from 0.94 to 1.00 (pooled 1.00, 95% CI 0.98-1.00). The PLR was 34.54 (95% CI 14.5-82.1), NLR was 0.24 (95% CI 0.16-0.36), and DOR was 204.92 (95% CI 76.8-546.6). $\chi 2$ values of sensitivity, specificity, PLR, NLR, and DOR were 44.99 (p=0.00), 5.45 (p=0.71), 34.54 (p=0.99), 40.55 (p=0.00), and 2.68 (p=0.95), respectively, suggesting heterogeneity among studies to some extent.

The Figure 3 showed the SROC curve plotting the true-positive against the false-positive rates of individual

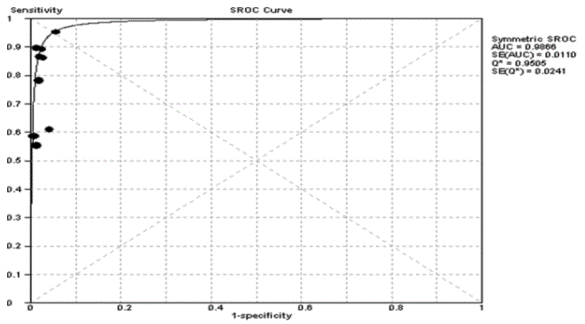

Figure 3. Summary Receiver Operating Characteristic (SROC) Curve of FISH Assay for the Diagnosis of MM. The size of each solid circle represents the size of each study included in the present meta-analysis. The regression SROC curve indicates the

studies. As a global measure of test efficacy we used the Q-value, the intersection point of the SROC curve with a diagonal line from the left upper corner to the right lower corner of the ROC space, which corresponds to the highest common value of sensitivity and specificity for the test. This point does not indicate the only or even the best combination of sensitivity and specificity for a particular clinical setting but represents an overall measure of the discriminatory power of a test. In the present meta-analysis, the maximum joint sensitivity and specificity of our study was 0.95 (the Q value), the AUC was 0.99, indicating a high level of overall accuracy.

Discussion

The diagnosis of MM is an important clinical challenge because the incidence of this high aggressive tumor is increasing, however, the limited biopsy material that lack definitive evidence of invasion and the lack of classic morphologic signs of malignancy with only subtle cytologic abnormalities or paucicellularity in cytologic examination make the definitive diagnosis of MM difficult (Pereira et al., 2006). To find a new and effective diagnostic tool for MM will be of great importance for its treatment and prognosis. The present meta-analysis investigated the overall diagnostic role of FISH assay in the differential diagnosis of MM with a high specificity 1.00 (95% CI 0.98-1.00), while the sensitivity was only 0.72 (95% CI 0.67-0.76) and with more variable than specificity. Our data indicated that FISH assay might be somehow helpful in confirming MM, but these assays maximize the specificity at the cost of sensitivity and have significant influence on clinical implications.

The SROC curve presents a global summary of test

performance and indicates the trade-off between sensitivity and specificity (Walter, 2002). Our meta-analysis based on SROC curve showed the maximum joint sensitivity and specificity was 0.95, and the AUC was 0.99, indicating a high level of overall accuracy, the FISH assay plays an important role in the diagnosis of MM. DOR, the ratio of the odds of FISH assay-positive test between patients with disorder and those without it, is another indicator of test accuracy which combines the data from sensitivity and specificity into a single number (Glas et al., 2003) The value of a DOR ranges from 0 to infinity, with higher values indicating better discriminatory test performance (higher accuracy). A DOR of 1.0 indicates that a test does not discriminate between patients with the disorder and those without it. In the present study, the DOR was 204.92 (95% CI 76.8-546.6), indicating that FISH assays seemed to be valuable in the diagnosis of MM. Because the SROC curve and the DOR are not easy to interpret and use in clinical practice, while likelihood ratios are considered more clinically meaningful, we also presented both PLR and NLR as our measures of diagnostic accuracy. A PLR value of 34.5 suggests that patients with MM have about 35-fold higher chance of being FISH assay-positive compared with patients without MM, it is helpful for the clinical practice. On the other hand, NLR was found to be 0.24 in the present meta-analysis. It means if the FISH assay result was negative, the probability that this patient has MM is 24%, which is not low enough to rule out MM. In addition, FISH test paly a prognostic role in the MM. In Chung's study (Chung et al., 2010), FISH status with clinical outcome showed that P16/CDKN2A deletion was associated with a worse outcome, with a 50% two-year survival for lack of p16/CDKN2A deletion versus 17% survival for patients with the deletion. This is consistent with previous studies identifying loss of p16/CDKN2A as a poor prognostic indicator (López et al., 2006; Dacic et al., 2008). To perform FISH test, MM patients will benefit from both diagnostic and prognostic aspects.

The present study suggests that FISH assay may be valuable for the diagnosis of MM, especially in the confirmation of MM, however, there are still several challenges exist. Firstly, although we made comprehensive search strategy, the screening, study selection, data extraction and quality assessment were done independently and reproducibly by two reviewers, there were only nine studies included, the limited patients numbers may have influence on the outcomes, further studies at a large scale may be needed to confirm the diagnostic role of FISH assay in MM. The second, due to the limited studies included, we did not use QUADAS scores to perform the meta-regression analysis to assess the effect of study quality on relative DOR of FIHS assay in the diagnosis of MM. And for the same reason, we could not explore whether or not study design such as blinded, cross-sectional, consecutive/random and prospective design affect the diagnostic accuracy, either. Last but not least, most studies used CDKN2A (p16), chromosome 9 as the probes in the FISH assay, however, up to now, there were no standard FISH assay probes for the diagnosis of MM, to develop a standard and commercial available FISH test is urgent.

To summarize, FISH plays an important role in the diagnosis of MM and is likely to confirm the diagnosis. Limited to the number and quality of current available studies, during clinic practice, the results of FISH assay should be interpreted in parallel with clinical findings and the results of conventional tests.

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