Editorial

Mesothelioma diagnosis and prognosis, are we moving beyond histology and performance status towards circulating biomarkers?

Oluf Dimitri Røe[1,2,3]

[1]Department of Clinical Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, NO 7491, Norway; [2]Cancer Clinic, Levanger Hospital, Nord-Trøndelag Hospital Trust, Levanger, NO 7600, Norway; [3]Department of Oncology & Clinical Cancer Research Center, Department of Clinical Medicine, Aalborg University Hospital, Aalborg, DK 9000, Denmark

Correspondence to: Oluf Dimitri Røe. Department of Clinical Research and Molecular Medicine, Norwegian University of Science and Technology, Prinsesse Kristinsgt. 1, Trondheim, NO 7491, Norway. Email: olufdroe@yahoo.no; oluf.roe@ntnu.no.

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An interesting overview and discussion of which circulating mesothelioma biomarkers are, and which may become clinically relevant, is presented by Huan H. Sun, Allen Vaynblat and Harvey I. Pass in "Diagnosis and prognosis—review of biomarkers for mesothelioma" (1).

Malignant mesothelioma is a cancer of the pleural lining in about 80% of cases, the lining of the peritoneum in 20%, and the tunica vaginalis testis in rare cases (2). Mesothelioma was almost unknown before the industrial use of asbestos. Due to the long latency from exposure to cancer of 20–60 years with a median of 40 years, there is still an increase of the disease in most countries where proper registration exists, even 30 years after banning asbestos. In the so-called BRICS countries, with the exception of South Africa that banned asbestos in 2008 (2), still until 2015, 2 million tons of asbestos was produced and/or consumed every year, and this world-wide epidemic will continue to increase for as long as asbestos is in use. This includes chrysotile asbestos or white asbestos, that is still promoted as safe in some of these countries (3). Importantly, for each asbestos-induced mesothelioma there are estimated six lung cancers (4) so, if there are 3,000 mesotheliomas annually in the US, there are 18,000, an incredible number of people that get lung cancer due to asbestos +/– cigarette smoking.

Mesothelioma has three main histological subtypes: the epithelioid which is the most common and has the highest median survival, the sarcomatous which has the lowest survival, and the biphasic type which contains both cell types. Throughout subtypes and stages, when standard chemotherapy is administered, median survival is 12–14 months, but one can see patients surviving for several years with multimodal therapy, including chemotherapy, surgery and radiotherapy, but also with chemotherapy alone (2,5). Surgery is still not the standard of care, especially after the MARS study. Some would not recommend surgery except in clinical trials (6,7). Pleurectomy-decortication seems to be the most favored type of surgery, currently (8). Still, non-invasive diagnostic, prognostic and predictive biomarkers are lacking from our daily clinical practice.

The authors of this review are deeply involved in mesothelioma biomarker research and know their field well. This review, for all practical purposes, describes biomarkers for malignant pleural mesothelioma (MPM). They discuss in a comprehensive way some of the most promising circulating or non-invasive biomarker candidates, the serum and plasma proteins mesothelin or soluble mesothelin-related proteins (SMRP), osteopontin, fibulin-3, high mobility group box 1 (HMGB1), the proteomic slow off-rate modified aptamer (SOMAmer) assay, microRNA and ratios of white blood cells, as lymphocyte-to-monocyte ratio (LMR) and neutrophil-to-lymphocyte ratio (NLR).

The authors believe that the survival of MPM patients has not improved using the well-known poor prognostic

factors, including poor performance status, non-epithelioid histology, male gender, elevated LDH, leukocytosis, thrombocytosis and anemia. This is consistent, since there are no definitive treatments for MPM, so that stratifying patients into different treatment modalities based on these factors does not cure these patients. Moreover, MPM is usually diagnosed at a stage where surgery is difficult and where earlier diagnosis could have improved survival prospects for more patients.

Non-invasive biomarkers for earlier detection and stratification of patients into more effective treatment regimens would be of high value as this disease will continue to plague people for many more decades.

Biomarkers of diagnosis

When speaking of diagnostic biomarkers one must emphasize that the gold standard for MPM diagnosis is by immunohisto- and cytochemistry. According to the current guidelines two positive and two negative antibodies are needed to establish a MPM diagnosis, where calretinin is the only of the important histological markers that currently has been evaluated as a future circulating diagnostic biomarker (9).

In contrast to lung cancer, early diagnosis in MPM is not recognized as helpful in increasing survival. Since most patients are diagnosed in advanced stages we do not actually know whether early diagnosis would impact survival. In a few cases identified with parietal pleura affection alone, median survival was 32 months while in case of affection of both parietal and visceral pleura, the survival dropped to 7 months, defined by the 7th IASLC staging system as T1a and T1b (10). The recent 8th IASLC staging, however, is based on the fact that one could not detect survival difference between these two groups, therefore MPM in either pleura is defined as (11) stage T1. Stage IA (T1N0M0) with no nodal affection has a 5-year median survival of 16% while for Stage IV the 5-year survival is 0% (12). Consequently, there is good reason to hope that early diagnosis my lead to better outcomes.

Mesothelin, or SMRP (soluble mesothelin-related protein) is a glycoprotein that is expressed on the surface of mesothelioma, ovarian, pancreatic and some other cancers, but exhibit very low or no expression in normal tissues, except in mesothelial cells (13). SMRP is currently the only FDA approved mesothelioma biomarker (14). In a key study by Robinson *et al.* in 2003, some individuals were found to have elevated levels in the serum several years before being diagnosed with MPM (11). Due to this finding, we conducted a study on pre-diagnostic serum 1–30 years before MPM diagnosis from the Janus Serum-bank, Oslo, but there was no difference in serum mesothelin levels between cases and controls (15). However, one could speculate that the reason could be the long median lag-time of 15 years from serum sampling to diagnosis. Subsequently, Creaney *et al.* found elevated mesothelin in 17 of 106 cases in pre-diagnostic serum samples, but overall this result could not justify a full-scale screening (16). Thus, for screening/early diagnosis mesothelin alone was not recommended.

However, mesothelin or SMRP could be helpful as an adjuvant diagnostic. In the study of SMPR in serum from 2003, a sensitivity of 84% for mesothelioma, and 100% specificity in differentiating mesothelioma from other pleural diseases, 95% against other lung cancers and 100% against apparently healthy subjects (11). In a subsequent meta-analysis of 16 studies where mesothelin was tested as a diagnostic, using the common diagnostic threshold of 2.00 nmol/L, the range of sensitivity and specificity varied from 19–68% and 88–100%, respectively (17).

Mesothelioma diagnosis can be delayed due to lack of histological or cytological findings. In such cases, an elevated serum mesothelin is a clear signal of malignancy. However, a negative mesothelin, which is the case in roughly 50% of mesothelioma patients is clearly no proof of being cancer-free.

Monitoring mesothelioma by CT scan is not easy due to the non-circular tumor growth pattern, and therefore modified RECIST-criteria are used for mesothelioma (12) When serum mesothelin is elevated before treatment, a further elevation or reduction in serum mesothelin is a clear indication of tumor growth, recurrence or regression [reviewed in (13)]. Therefore, mesothelin, like other tumor monitoring markers, like CEA or CA15-3 is useful only in patients where there is an elevation before treatment.

Mesothelin levels are also elevated in pleural fluid of patients with MPM compared to patients with pleural metastasis of carcinomas or benign pleural lesions. Pleural fluid mesothelin concentrations are significantly higher in epithelioid mesothelioma compared to the sarcomatous type. It is of importance that pleural fluid mesothelin measurement has higher sensitivity than cytological examination—71% *vs.* 35% and a specificity of 89% *vs.* 100% respectively (9).

Osteopontin is an extracellular cell adhesion protein that mediates cell-matrix interaction and cell-signalling

via interaction with integrin and CD44 receptors. In-vitro studies showed that osteopontin was overexpressed in asbestos exposed cells and was also found as well as in asbestos-induced carcinogenesis in a rat model. In the first large study on plasma of asbestos exposed, non-cancer individuals and individuals with mesothelioma, plasma osteopontin had an impressive sensitivity and specificity of 77.6% and 85.5%, respectively (18). Further studies failed to confirm those results, and therefore this biomarker was discontinued as a candidate diagnostic marker.

Slow Off-Rate Modified Aptamers (SOMAmers) are short, single-stranded deoxynucleotides that bind molecular targets, and panels of proteomic targets have been used in studies for biomarker discovery. A 13-biomarker panel was found to detect MPM in asbestos-exposed individuals with overall accuracy of 92% and an AUC of 0.95; an excellent potential tool for early detection of MPM (19). However, after its publication in 2012 there have not been any follow-up or validation studies published, other than a study design report, the DIAPHRAGM study, that aims to study both the 13-protein panel and fibulin-3. Results from this study are awaited (20).

High-mobility group box 1 (HMGB1) protein, is a ubiquitous chromatin component expressed in nucleated mammalian cells. HMGB1 is a multi-potent protein, a mediator of inflammation and has been shown to play a role in transcription regulation of several cancer genes, including BRCA1, E-selectin, TNF-α and the insulin receptor (21). Recent studies have shown that asbestos exposure leads to primary human mesothelial cell necrosis. This results in release of HMGB1, binding to its main receptor, causing Nalp3 inflammasome activation and IL-1b secretion. The hyperacetylated HMGB1, showing a 100% sensitivity and specificity for diagnosis of MPM, seems to be the most powerful circulating single molecule diagnostic to date and the results of independent validation are awaited (22).

Biomarkers of prognosis

Mesothelin in serum and pleural fluid has shown a consistent value as an adjuvant diagnostic tool, however, as shown in repeated studies, as a prognostic marker it has no value (23).

Osteopontin was found to be a potential prognostic marker and even improve the EORTC clinical prognostic index. In a study of pre-operative plasma osteopontin by Pass *et al.*, it was found that log-osteopontin, EORTC clinical prognostic index, and hemoglobin were independently significant predictors. Including log-osteopontin to the entire prognostic model improved the C-index significantly, from 0.718 (0.67–0.77) to 0.801 (0.77–0.84) (24). This is actually an interesting route to explore, since biomarkers by themselves in general (23) do not seem to be a strong enough guide to clinical decisions.

Fibulin-3 is encoded by the epidermal growth factor, containing fibulin-like extracellular matrix protein 1 gene (EFEMP1). Fibulin-3 plays multiple roles in cell morphology and growth, adhesion and motility, especially with regard to tumorigenesis. Fibulin-3 is not found only in tumors, but also in plasma and pleural effusions and was evaluated as a diagnostic marker, in addition to fibulin-3 IHC expression in tumor tissues. Pass *et al.* found that plasma fibulin-3 could distinguish very well between asbestos exposed non-cancer subjects and mesothelioma patients and thus could be a new diagnostic marker. However, independent studies could not validate this finding, but have established this marker as a solid prognostic marker; when elevated in pleural fluid or in plasma, survival is significantly lower (25,26).

In their independent analysis Creaney *et al.* found that MPM patients with effusion fibulin-3 levels below the median (446 ng/mL, range, 204–1,408) survived significantly longer than those with levels above the median (14.1 *vs.* 7.9 months, P=0.012) (26).

Elevated serum HMGB1 was found to be a potential prognostic biomarker of MPM already in 2013 and is among the good candidate molecules for prognostication (27). Interestingly, Wu *et al.* did a meta-analysis of its value in different cancer types, and high HMGB1 protein in serum and tissue seems to be a ubiquitous negative marker for cancer survival, while HMGB1 mRNA is not (28).

MicroRNAs regulate transcription of DNA by binding to messenger RNA (mRNA), degrading mRNA and silencing target genes. Single miRNAs can regulate tens to hundreds of genes and affect cell growth, differentiation and apoptosis. Several studies have been conducted to discover potential diagnostic patterns, with mixed success. However, for predicting survival after mesothelioma surgery, Pass *et al.* already in 2010 found a single microRNA, miR-29c (29) and later Kirschner *et al.* in 2015 identified a six-microRNA signature that could separate groups of high and low survival (OS 21.6 *vs.* 9.1 and 15.4 *vs.* 6.5 months, respectively) (30). The results indicate that it may be worth studying circulating microRNAs as biomarkers for MPM surgery, and to avoid over-treatment; such biomarkers are of high priority.

The review also goes through the literature on ratios of various common haematological factors as LMR, NLR and platelet-to-lymphocyte ratio (PLR) and their potential as prognostic markers. Some of these ratios have been found prognostic in several cancer types e.g., gastric cancer (31). The NLR could not be verified in an independent study (32). The most promising study is probably a retrospective study of 150 patients where patients with LMR greater than 2.74 had a median overall survival of 14 months versus 5 months in patients with lower LMR and was confirmed as an independent prognostic marker (33).

Finally, the authors mention integrin, BAP-1, calretinin (see above) (34), caveolin-1, and P16-CDKN2A as promising new markers that still need much work in order to become established clinical markers.

More candidate markers

The review has omitted discussion of some important old and some new candidates. One of the most studied and simple biomarkers of prognosis is serum lactate dehydrogenase (LDH). In a meta-analysis of an impressive 1,977 patients from nine studies, the eight showed the value of LDH as a predictor of poor survival (35). Recently the so-called Controlling Nutritional Status Score (CONUT) calculated by the serum albumin and total cholesterol concentration was launched as a prognostic for MPM, where patients with a high CONUT score had a poorer overall survival ($P<0.001$) and poorer disease- or progression-free survival ($P<0.001$) (36). These last two examples show how biomarkers that are not obviously related to tumor growth actually can be used for prognostication. Although there is still a long way to go to validating all these markers prospectively, LDH and CONUT would have a significant advantage, in that they are blood tests one can do in any laboratory in the world and potentially get a picture of the patient's prognosis.

One of the newest up-and-coming biomarkers is mesothelioma-specific protein transcript variants of ecto-nicotinamide adenine dinucleotide oxidase disulfide-thiol exchanger 2 (ENOX2); this could be a true early diagnostic marker in the blood (37). The test has already been commercialised as the ONCOblot tissue of origin cancer detection test but needs validation. Midkine (MDK) is a heparin-binding growth factor expressed during embryogenesis but is down-regulated to an insignificant level in healthy adults. The MDK protein promotes cell growth, migration, and angiogenesis, in particular during tumorigenesis. High serum MDK is a true independent negative prognostic factor in mesothelioma, in contrast to mesothelin which has no prognostic value (38).

In summary, in spite of several candidate blood and pleural fluid biomarkers for MPM diagnosis and prognosis, only mesothelin is an FDA approved, commercially used clinical biomarker (14). As an adjuvant for diagnosis and monitoring it appears at least as useful as "classical" tumor markers e.g., CEA for colon cancer and CA-15-3 for breast cancer. Several of the above-mentioned markers have substantial potential and merit validation, and combination of molecular with clinical markers has shown promise. Since surgery seems to play a role in subsets of patients, tools as e.g., microRNAs to select the patients that will benefit is crucial. Biomarkers for predicting response to systemic therapy, including chemotherapy and immunotherapy should be of high importance, as more than half of the patients are non-responders, only left with the toxicities. Finally, the role of biomarkers as drug targets may be very important, as several studies on mesothelin-based treatments as well as microRNA mimic of miR16 showed impressive responses and manageable side effects (39,40). Moreover, we anticipate the era of immunotherapy to impact MPM and the accompanying "brand new" biomarkers which will be discovered in years to come.

The man-made cancer epidemic of mesothelioma is on the rise and will be for many decades to come, and therefore more research on screening, diagnosis, monitoring and treatment of our patients is of urgent need.

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Footnote

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