Cancer Antigen 125 As a Biomarker in Peritoneal Dialysis: Mesothelial Cell Health or Death?

Editor:

It is well known that serious damage of the peritoneum, used as a dialysis membrane, can be present without severe abnormalities in the commonly used parameters of peritoneal transport. The additional use of biomarkers originating from peritoneal tissues is therefore an attractive and potentially important area for exploration.

The glycoprotein cancer antigen 125 (CA125) is such a biomarker. Evidence has been obtained, mainly from studies by my group, that it represents peritoneal mesothelial cell mass in stable PD patients. That concept has recently been challenged by Cheema and Bargman (1). Regrettably, their review is rather unbalanced, and some relevant references are not mentioned. For instance, the first two papers on CA125 secretion by peritoneal mesothelial cells are not quoted, although they provide important information (2,3). The merely clinical study by Koomen *et al.* (2) in

continuous ambulatory peritoneal dialysis (CAPD) patients showed a relationship between the number of mesothelial cells and the CA125 concentration in effluent from long nightly exchanges, although the values had no normal distribution. Furthermore, the authors found higher concentrations of CA125 in the supernatant when more mesothelial cells were brought into culture. The authors also showed that 50%–80% of cytokeratin-positive cells in effluent stained positive for CA125.

The *in vitro* study by Zeillemaker *et al.* revealed that CA125 is preferentially secreted from the apical surface of mesothelial cells (3). That study also reported higher CA125 secretion after stimulation with supraphysiologic dosages of tumor necrosis factor α and interleukin 1 β (IL-1 β). The study by Visser *et al.* (4), in another population of CAPD patients, confirmed the previously reported relationship between the number of mesothelial cells and the CA125 concentration in effluent. Additionally, those authors reported no effect on CA125 secretion by cultured mesothelial cells when quantities of tumor necrosis factor α and IL-1 β less excessive than those in the previous study were used. However, the quantities applied stimulated the production of IL-6 and IL-8 by mesothelial cells in a dose-dependent manner. Exposure to transforming growth factor β was not associated with enhanced CA125 production.

The combination of the effect on CA125 release of the number of mesothelial cells brought into culture and the fact that CA125 release could not be stimulated with cytokines—as also confirmed in a more recent *in vitro* study using material obtained from non-peritoneal dialysis (PD) patients (5)—leads me to contend that effluent CA125 in stable PD patients can be considered a marker of mesothelial cell mass when used for follow-up in individual patients.

Cheema and Bargman use the aforementioned study by Breborowicz *et al.* (5) to argue that CA125 is not a reflection of mesothelial cell mass. They ignore a number of weaknesses in that paper. First, it is a cross-sectional analysis in omental tissue from patients who are only known not to have been on PD and to have required abdominal surgery for unknown reasons. Second, approval of the study by a medical ethics committee is not even mentioned in the paper. Third, CA125 in peritoneal effluent from PD patients is well known to show marked interindividual variability, because not all mesothelial cells express CA125 to the same extent (2,6). It is therefore quite logical to assume that cultured mesothelial cells from a heterogeneous group of non-PD patients will demonstrate similar variability in CA125 expression. In such a situation, a cross-sectional analysis comparing numbers of cultured mesothelial cells that have never

been exposed to PD solutions and CA125 concentration in culture supernatant cannot be used as an argument to reject the results of our studies. The cross-sectional design and lack of clinical information might also explain why CA125 concentration in culture supernatant was related to the age of the donors.

Another issue in the review by Cheema and Bargman is the presence or absence of a relationship between effluent CA125 and the transport of low molecular weight solutes. Initially we found no such relationship (7), which seems to contrast with results published by others (8). It is now evident that a relationship is present only during the first 2 years of PD, and not in subsequent years (9). The explanation might be that mesothelial cells secrete a large number of vasoactive substances—at least *in vitro*—that may influence the number of perfused peritoneal microvessels. That theory is supported by the observational study of van Esch *et al.*, who showed, in incident nondiabetic PD patients who had never experienced peritonitis, that solute transport is related to effluent CA125 and that the relationship could be explained by vascular endothelial growth factor, not by IL-6 (10). The combination of a decline in mesothelial cell mass with time on PD and an increase in solute transport because of neoangiogenesis is the most likely reason for the disappearance of the relationship.

Peritonitis is also discussed in the review. The temporary elevation of CA125 levels during peritonitis (11) is easily explained by the effects of desquamation of dead mesothelial cells or by lysis of mesothelial cells, as occurs after *in vitro* exposure to a Triton (Dow Chemical, Midland, MI, USA)-containing solution (4). That explanation accords with the very low number of mesothelial cells found in effluent during peritonitis (12) and has nothing to do with the somewhat high number of dead mesothelial cells reported in stable patients, although without an effect on CA125 (8).

Perhaps the most important topic discussed by Cheema and Bargman is an interpretation of the increase in effluent CA125 concentration that has been found in all studies in which prevalent PD patients were switched from conventional dialysis solutions to biocompatible solutions. The most likely explanation is the induction of an increase in mesothelial cell mass because of less mesothelial degradation. The review authors suggest an opposite interpretation. They postulate that biocompatible solutions are harmful to the mesothelium, because they induce a state of inflammation that could lead to mesothelial cell death. However, they ignore the results of the sparse animal experiments that have been performed (13,14) and an important clinical observation (15).

The study by Hekking *et al.* (13) in rats was done after 9 – 10 weeks of daily peritoneal exposure to either a conventional or a commercially available bicarbonate/lactate-buffered solution. It was evident that the bicarbonate/lactate solution caused less inflammation and better preserved the mesothelium. Similar experiments, but with a daily exposure duration of 20 weeks, showed a marked reduction in the number of peritoneal blood vessels, a decreased amount of peritoneal fibrosis, and an absence of inflammation in the animals receiving bicarbonate/lactate solution compared with those receiving conventional solution (14). Expression of CA125 could not be assessed in any rat study, because the antibody used for its determination is generated in mice and shows cross-reactivity in rats. The duration of exposure in the two studies was much longer than the 4 weeks usually used, but still short for humans; however, the lifetime of a rat is usually 2.5 years, which means that, by extrapolation, the exposure used would equal a period of 7 – 11 years in humans.

The clinical observation from Japan in a PD patient treated with biocompatible solution for a period of only 4 years showed almost no histologic differences from a peritoneal biopsy obtained at the start of treatment—and especially no signs of inflammation (15). A more recent study, also from Japan, showed that patients treated with a biocompatible dialysis solution had less peritoneal fibrosis and vascular sclerosis than were found in patients treated with conventional solutions, but no mention was made concerning signs of inflammation (16).

I think that the conclusion is evident. Effluent CA125 in stable PD patients without acute peritonitis is a marker of mesothelial cell mass, but has large inter-individual variability. Serial measurements over time can be used for assessment of peritoneal mesothelial mass in individual patients. The increase of CA125 in prevalent patients after a switch to biocompatible solution does not point to peritoneal inflammation, but is likely to indicate reduced dialysate-induced degradation. Whether that effect persists with longer PD duration is unknown.

DISCLOSURES

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Re: Cancer Antigen 125 As a Biomarker in Peritoneal Dialysis: Mesothelial Cell Health or Death?

Editor:

I sincerely thank Dr. R.T. Krediet, a giant in the field of peritoneal physiology and pathophysiology, for responding to our opinion paper on cancer antigen 125 (CA125) (1).

Dr. Krediet was co-author of the paper by Koomen *et al.* (2) demonstrating that the CA125 in dialysis effluent must originate from within the peritoneal cavity, and correlating CA125 with the number of mesothelial cells *in vivo* and *in vitro*. However, in that paper, the authors quote another study from a decade earlier (3) that demonstrated that CA125-positive mesothelial cells are indeed located in the peritoneum, but especially in areas of inflammation and adhesions. They also note that CA125 levels are high in patients with malignant ascites and ascites associated with liver disease. So even in these early reports, it is not clear that effluent CA125 reflects a state in which all is well with the peritoneum.

Dr. Krediet further quotes the *in vitro* study by Zeillemaker *et al.* (4) that used human omentum [an approach that he cites as a weakness of the Breborowicz study (5)] and that found that tumor necrosis factor α , interleukin 1 β , and *Escherichia coli* lipopolysaccharide lead to increased CA125 secretion. Those results would be supportive of our own hypothesis that this biomarker might not necessarily reflect mesothelial health and mass, but instead might be a sign of intraperitoneal inflammation.

The hypothesis that a higher concentration of CA125 in peritoneal dialysis effluent may reflect an inflammatory state, such as peritonitis, is countered by Dr. Krediet's

alternative suggestion that elevated CA125 is the result of desquamation of dead or lysed mesothelial cells. However, that suggestion contradicts the statement in his own paper contending that CA125 in effluent cannot be explained by drained mesothelial cells. Indeed, his group states that it is "unlikely that more than 1% of the CA125 in the dialysate is produced by the mesothelial cells in the effluent" (2).

The final issue concerns pH-neutral, so-called "bio-compatible," peritoneal dialysis solutions. Dr. Krediet quotes his own animal studies finding a marked reduction in peritoneal microvessels when the animals received bicarbonate/lactate dialysate (6). It would be difficult to extrapolate that finding to the many human studies in which peritoneal permeability increases, rather than decreases, with the use of pH-neutral solutions. Indeed, in the recent Japanese study that Dr. Krediet cites, histology examination detected more angiogenesis and new vessel formation in patients using pH-neutral solution than in patients using conventional dialysate (7).

Clearly animals are animals, humans are humans, and many factors are at play in the response of the peritoneum to the onslaught of dialysis fluid. The purpose of our paper was to highlight the uncertainty surrounding this subject and to give pause to those who reflexively assume that an increase in effluent CA125 means only good things for the peritoneal membrane.

DISCLOSURES

JMB has been a consultant for Amgen and Baxter Healthcare, and is in the speakers' bureau for Amgen and DaVita Healthcare Partners.

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