New Ultrasensitive Assays Facilitate Studies on the Role of Human Glandular Kallikrein (hK2) as a Marker for Prostatic Disease

Prostate cancer is the most common non-skin cancer in men, and it is an important cause of morbidity and mortality. The growth rate of prostate cancer is unusually slow. It has been estimated that the tumor starts developing at 20–30 years of age and reaches a detectable stage 30–40 years later *(1)*. This estimate is based on the doubling time of the serum concentrations of prostate-specific antigen (PSA), ~2 years *(2)*, and the prevalence of high-grade prostatic intraepithelial neoplasia and microscopic cancers in prostates removed at autopsy *(3, 4)* in various age groups. Studies on archival serum samples from men who later developed prostate cancer have shown that the serum concentrations of PSA begin increasing 5–10 years before clinical presentation *(2, 5)*. When serum PSA exceeds the commonly used cutoff (4 μg/L), the tumor is mostly organ-confined and potentially curable. Therefore, PSA-based screening and case finding is now increasingly used for early detection of this disease *(6)*. However, the value of this screening is debated because the treatment causes morbidity *(7)* and, although recent data suggest that screening reduces prostate cancer mortality *(8)*, the long-term effect on mortality and quality of life remains to be demonstrated. When prostate cancer is treated without curative intent, the median disease-specific survival is 17 years *(5)*. Thus, it will not be possible to evaluate the long-term effects of screening for another 5–10 years, but it is hardly possible to reverse the trend to screen for prostate cancer until then. However, as laboratorians, we can help to reduce the negative effects of screening by developing better screening tools.

Currently, the main screening tool is serum PSA, which suffers from low specificity *(6)*. Approximately two-thirds of the increased PSA values in men over 50 years of age are caused by benign prostatic hyperplasia. The number of false-positive results can be reduced by ~30% by measurement of the proportion of two major forms of PSA in plasma, free PSA and PSA in complex with α_1-antichymotrypsin (PSA-ACT) *(9, 10)*. However, further improvement is desirable. The two new assays for human glandular kallikrein (hK2) described in this issue are the result of intense developmental work that has combined sophisticated molecular biology with antibody and assay technology *(11, 12)*. They may represent an important step forward in the search for better diagnostic tools for prostate cancer.

The expression of hK2 mRNA in the prostate was demonstrated ~10 years ago *(13)*. Although the abundance of hK2 mRNA in prostatic tissue is only slightly lower than that of PSA, it has been extremely difficult to detect hK2 in seminal fluid, which contains PSA at concentrations that are approximately one million-fold higher than those in serum *(14)*. Therefore, an antigen for immunization has not been obtained from natural sources, and the first specific assays were developed with antibodies raised against synthetic peptides selected to be specific for hK2. These assays, however, were not sensitive enough for clinical work *(15)*. Another approach has been to utilize the homology between hK2 and PSA to measure hK2 with antibodies to PSA. Approximately one-third of the antibodies raised against PSA cross-react with hK2, but the use of PSA-specific antibodies to block the binding of PSA to cross-reacting antibodies that detect hK2 has enabled the development of sensitive and specific hK2 assays *(16)*.

The methods described in this issue are based on antibodies raised against recombinant hK2, and it is interesting to compare the first results obtained with three principally different assays described thus far. The detection limits of the assays are fairly similar, with "biological" detection limits of 4–50 ng/L, which are sufficient for the detection of hK2 even in men without prostatic disease. The results from the studies of Klee et al. *(11)* and Kwiatkowski et al. *(17)* are similar, with serum hK2 concentrations that are ~1–3% of those of PSA. Clearly higher concentrations were reported by Black et al. *(12)*, i.e., the ratio of hK2 to PSA was nearly 10-fold higher. Interestingly, this group used one antibody specific for hK2 together with a PSA antibody, whereas Klee et al. *(11)* used two hK2-specific antibodies. The reasons for these discrepancies in serum hK2 results are not clear. Both groups used the same calibrator, consisting of recombinant hK2, but they used hK2 antibodies that were different, albeit produced by the same group *(11)*.

There are several potential explanations for the discrepancies in the hK2 concentrations measured: differences in epitope specificity in combination with differences between recombinant hK2 and plasma hK2, a variable nonspecific background, and different abilities to detect complexed forms of hK2. The assay of Klee et al. *(11)* has a minimal cross-reaction (3%) with hK2-ACT, whereas that of Black et al. *(12)* detects some complexes, which however, do not seem to contribute much to the serum concentrations of hK2 in the samples studied. However, the concentrations of PSA and hK2 in the samples were very high, and they may not be typical. Furthermore, the cross-reactivity of hK2 complexes in this assay is not known *(12)*. Much of the free hK2 in serum has been shown to consist of the proenzyme form (prohK2), but little is known about hK2 complexes. In seminal plasma, part of the hK2 is complexed with protein C inhibitor. However, when added to plasma, hK2 reacts most rapidly with α_2-macroglobulin, but it also reacts with C1-inactivator and probably with other inhibitors, which have not yet been positively identified *(18)*. The fact that the concentrations of complexed PSA are higher than those of free PSA *(9)* does not necessarily mean that the same is true for hK2. For example, the concentrations of free trypsinogen in serum exceed those of trypsin complexes *(19)*.

It is obvious that much work needs to be done before the clinical utility of hK2 can be evaluated. The limited

clinical information presented in the two studies in this issue does not suggest that hK2 is as useful as PSA. However, the key question is not whether hK2 can replace PSA, but rather whether the combined use of these markers can provide additional clinical information. The study of Kwiatkowski et al. *(17)* actually suggests that this may be the case. hK2 in combination with free and total PSA improved the cancer specificity in the restricted PSA concentration range of 4–10 μg/L. However, the number of patients was small, and further studies are needed before any certain conclusions can be drawn.

When evaluating the use of markers for prostate cancer screening, it is important to recognize that with a PSA-based screening, we pick out only a minor portion, ~10%, of the tumors that can be detected microscopically *(1)*. At the moment, we do not know whether these are the most urgent cases and whether some could be monitored with watchful waiting. However, once the PSA value exceeds 4 μg/L, it is common to perform prostate biopsies, only one-third of which will reveal carcinoma. It would be very valuable to have serum markers with better cancer specificity and with the ability to preferentially detect aggressive tumors. On the tissue level, the expression of hK2 is higher in tumors than in healthy epithelium *(20)*. This suggests that hK2 might be useful for evaluation of tumor aggressiveness. Although it is unlikely that a serum test would provide very reliable information in this respect, even a moderate improvement is valuable. Recent data suggest that a low percentage of free PSA is associated with high-grade tumors *(21)*; if hK2 would provide additional information of this type, the extra costs of performing another serum test could be justified. However, hK2 is not the only new potential marker. Methods for assay of complexes between PSA and α_1-protease inhibitor *(22)* and α_2-macroglobulin have been developed recently, and the first studies suggest that they also may provide additional diagnostic value *(23)*.

Interpretation of results from multiple analytes easily becomes complicated unless we use appropriate methods to evaluate the combined impact of various variables. Logistic regression is a well-established method that is gradually gaining popularity. Neural networks serve the same purpose and may in some cases be a better alternative *(24)*. An additional advantage of these methods is that they facilitate evaluation of the relative value of various diagnostic procedures. Thus, they help us not only evaluate new methods, they also help identify new tests that replace established ones. Acceptance of this approach probably requires that the laboratorian assume a more active role in the diagnostic process, which in any event is a goal worth pursuing.

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