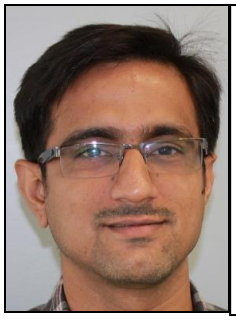
Clinical Chemistry

A Nanoparticle-Lectin Immunoassay Improves Discrimination of Serum CA125 from Malignant and Benign Sources



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Guest: Dr. Kamlesh Gidwani is senior researcher of Molecular Diagnostics in the Department of Biochemistry, Division of Biotechnology, at University of Turku, Finland.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Cancer antigen 125, or CA-125, is the most commonly used biomarker for epithelial ovarian cancer diagnostics and follow-up. However, current immunoassay methods lack specificity, and that increased CA-125 values are also detected in healthy controls and in benign diseases.

Because CA-125 is known to be differentially glycosylated in ovarian cancer versus benign cases, a group of researchers aim to exploit this as a way to improve the specificity of CA-125. The October 2016 issue of *Clinical Chemistry* included an article describing how this concept could help reduce the false positive rates of conventional cancer antigen 125 immunoassays. We are joined by this article's first author, Kamlesh Gidwani, for this podcast.

Dr. Gidwani is senior researcher of Molecular Diagnostics in the Department of Biochemistry, Division of Biotechnology, at University of Turku, Finland. His work is focused on cancer-specific lectin nanoparticle assay development.

And doctor first off, tell us about the lectin nanoparticle concept.

Kamlesh Gidwani: Well, lectins are carbohydrate-binding proteins and have been used to demonstrate glycosylation differences in glycoprotein derived from malignant and benign tissues. In our study, we used europium-doped polystyrene nanoparticle which are around 97 nanometer in diameter and doped with thousands of fluorescent europium-chelate-doped. Lectins were conjugated on the surface of this nanoparticle and were used for detection of glycoprotein captured by immobilized antibodies.

Bob Barrett: And what makes this concept better than conventional cancer antigen 125 immunoassays?

Kamlesh Gidwani: The great majority of traditional tumor markers, including CA-125, are glycosylated proteins. The conventional CA-125 immunoassays based on two monoclonal antibodies which recognize different protein epitopes and early record serum CA-125 is also found in patients suffering from endometrial cyst, liver diseases, and ovarian cyst. And this approach fails to disconnect cancer CA-125 from non-cancer CA-125. And due to this poor clinical specificity, isolated measurement of CA-125 is not recommended for ovarian cancer screening. And it is reported that serum CA-125 from ovarian cancer patients have different glycans structure compared to the benign conditions such as an endometrial cyst. And there are no commercial diagnostic kits, which detect the altered glycans on ovarian cancer serum CA-125, while our new lectin nanoparticle concept can specifically recognize the ovarian cancer specific glycans on serum CA-125. Besides being analytically highly sensitive, its reactivity with CA-125 from non-malignant sources was divisibly reduced or abolished.

Bob Barrett: And how does this application differ from previous uses of lectins?

Kamlesh Gidwani: Several studies have reported the use of lectin in cancer diagnostics, mostly using histochemistry and various lectins are reported combined to breast cancer cells in tissue section. For example, human lectin MGL, which is macrophage galactose type lectin, is also used for detection of glycans in section from formalin-fixed, paraffin-embedded cancerous mammary tissue.

The performance of lectin-based assay in serum sample has been affinities; therefore the limit of detection for lectin assay is frequently insufficient to enable use for early detection.

In our new assay concept, lectin nanoparticle strongly binds to specific glycans of the immobilized glycoprotein. The highly-improved analytical performance of the method is due to signal amplification by the 30,000 europium-chelate factor within the nanoparticle and to the avidity effect created by high density of immobilized lectins on the particle. And the europium nanoparticle-assisted lectin technology thus enabled the construction of a simple rapid two-step protocol suitable for sensitive glycan profiling for glycoproteins.

Bob Barrett: Doctor, there is interest in population-based screening for ovarian cancer, but one of the issues to date is related to the poor specificity of the available measurement methods for cancer antigen 125. How do you see your new test concept impacting this problem?

Kamlesh Gidwani: It's true. And it is well-known that cancer diagnostics at an early stage with sensitive and accurate biomarkers is a key to successful cancer treatment. And such biomarkers are especially important for cancer which remains asymptomatic until disseminated stage, where curative response can rarely be achieved and ovarian cancer is a good example of this.

Recently, Ian Jacob and his group published a large prospective ovarian cancer screening study in United Kingdom, which is called UKCTOCS, in *Lancet,* where they combined ultrasound and serial CA-125 measurement. The results were promising in the sense that they showed that this screening strategy may reduce disease mortality. But of course a more specific CA-125 test, this would reduce the number of false positive being subjected to invasive follow-up procedures and remove the need for serial measurement. We also measured CA-125 lectin MGL nanoparticle concentrations in sequential serum sample of 27 patients with high-grade serous ovarian cancer to well work the assay ability to detect disease recurrence. The relative CA-125 MGL nanoparticle values showed earlier increase in 37 percent patients with epithelial ovarian cancer with progressive disease, and higher fold-increase in 30 percent of patients, and this is suggesting better earlier detection of disease progression in two-thirds of patients. And our new assay construct could allow the clinician much earlier for alternate treatment.

So based on the improved differentiation of benign and malignant conditions at low marker confrontation, and earlier detection for ovarian cancer progression, we hypothesized the new test method may have potential for earlier detection for epithelial ovarian cancer. We hope to proceed with such studies in a screening setting shortly.

Bob Barrett: Many if not most of existing tumor markers are glycoprotein hormones. Do you think there is potential for this concept to be applied more broadly?

Kamlesh Gidwani: Yes, it's true that many tumor markers are glycoproteins and appear widely to have aberrant glycans, and there have been many studies claiming the detection of aberrant glycans with the use of lectins. The success in practice has been very limited, and we feel that the main reason for this is the poor affinity of the lectin used. Certainly, we feel that this new concept is likely to be very effective in developing cancer-specific assays for other common tumor markers to be used in serum, plasma, and urine. Henna Kekki, a researcher in our group, recently reported a lectin aleuria aurantia, when conjugated with this nanoparticle could discriminate PSA (prostate specific antigen) derived from prostate cancer cell line LNCaP and non-cancer origin seminal plasma PSA. She detected a significant increase in

the PSA fucosylation in prostate cancer tissue compared to benign tissue and in urine from prostate cancer patient compared to the benign patient.

Bob Barrett: Finally doctor, why did you select this particular detection method, and do you see a need or potential for achieving similar results using other reporter systems?

Kamlesh Gidwani: Well, the basic concept behind the uses of europium nanoparticle is to provide the multivalency of cancer specific lectin, in other words improving the affinity to avidity effect. So any particle like colloidal gold nanoparticle, silver nanoparticle, up converting phosphorus, quantum dots, carbon nanoparticle, and many others will certainly improve the binding affinity of lectin with its specific sugar moieties on immobilized glycoprotein. A particular advantage of using europium-doped nanoparticle is the enormous inherent potential for signal amplification. A glycovariant sub-form of a tumor marker may represent a small section of total concentration of marker calling for detection techniques of executing sensitivity.

Bob Barrett: Dr. Kamlesh Gidwani is senior researcher of Molecular Diagnostics in the Department of Biochemistry, division of Biotechnology at the University of Turku in Finland. He's been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening!