Research Highlights

Highlights from the latest articles in biomarkers

Vascular dysfunction biomarkers are elevated in children with HIV

Evaluation of: Miller T, Somarriba G, Orav EJ *et al.*: Biomarkers of vascular dysfunction in children infected with human immunodeficiency virus-1. *J. Acquir. Immune Defic. Syndr.* (2010) (Epub ahead of print).

In developed countries, children with HIV now live longer than in the past. Multiple studies in adults have demonstrated a relationship between HIV and increased vascular dysfunction [1]. The association of HIV with vascular dysfunction in children has been difficult to determine because clinical evidence of vascular disease is far less common in children than in adults. The questions that the researchers in this study asked were whether biomarkers associated with vascular dysfunction were present in children with HIV, how treatment with highly active antiretroviral therapy affected biomarker expression and what the potential long-term consequences were.

The study evaluated cases enrolled in an NIH-supported study on cardiovascular risk in children with HIV, with controls being children and youths without the disease. The investigation evaluated nine biomarkers associated with vascular dysfunction: C-reactive protein, IL-6, monocyte

chemoattractant protein (MCP)-1, soluble ICAM-1, fibrinogen, P-selectin, soluble VCAM-1, E-selectin and leptin.

The investigators found that children with HIV had higher levels of five of the biomarkers (soluble ICAM-1, soluble VCAM-1, MCP-1, IL-6 and fibrinogen). A higher level of MCP-1 was associated with greater waist:hip ratio, C-reactive protein with increased body fat, and MCP-1, C-reactive protein and IL-6 with increased severity of HIV. The authors admit that, similar to other studies of cardiovascular risk in children, associations with risk may not equate to adverse outcomes and years of additional follow-up of the children are required to determine if the biomarkers are truly associated with risk of future disease. Nonetheless, the observations of increased expression of multiple vascular dysfunction biomarkers in children with HIV suggests that the children should be followed so that, if validated, these biomarkers could be used to identify individuals who would benefit from risk-reduction strategies.

Reference

- 1 Mu H, Chai H, Lin PH *et al.*: Current Update on HIV-associated vascular disease and endothelial dysfunction. *World J. Surg.* 31, 632–643 (2007).

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Circulating proteins predict liver fibrosis in hepatitis C infection

Evaluation of: Ho A, Cheng C, Lee S *et al.*: Novel biomarkers predict liver fibrosis in hepatitis C patients: α 2 macroglobulin, vitamin D binding protein and apolipoprotein A1. *J. Biomed. Sci.* 17, 58 (2010).

Liver fibrosis is a common result of hepatitis C infection, which can lead to cirrhosis and/or hepatocellular carcinoma. There are four stages of fibrosis of increasing severity from F1 to 4, with F4 indicating cirrhosis. The early detection of liver fibrosis can mitigate the potential damage from the disease. The gold standard

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for diagnosing fibrosis is a liver biopsy, which has attendant risks. Noninvasive approaches to assess the liver in patients with hepatitis C infection have been evaluated, including elastography (a technique that combines 1D ultrasound and low-frequency elastic waves to measure elasticity) and circulating proteins. The authors evaluated serum from individuals at various stages of fibrosis, as well as controls without known cirrhosis, to identify predictors of fibrosis that might ultimately be used to minimize the need for liver biopsy.

Serum samples were separated using 2D difference gel electrophoresis into individual proteins and peptides. Three spots were identified as upregulated (α 2 macroglobulin [A2M]) or downregulated (vitamin D binding protein [VDBP] and ApoA1) using mass spectrometry, based on the presence or absence, and severity of fibrosis.

The investigators found that median A2M levels were lower in subjects without fibrosis than in those with either mild

or severe fibrosis. Median levels of VDBP and ApoA1 were lower in subjects with severe fibrosis compared with those with out or with mild fibrosis. It has previously been reported that A2M and ApoA1 are associated with liver fibrosis. The association of VDBP appears to be a novel finding. Nonetheless, the information provided by A2M, rather than VDBP or ApoA1, is likely to have greater clinical relevance, as it increases in expression with disease progression, which is easier to assess in a clinical setting than decreased expression. Moreover, the investigators report a difference in A2M expression between subjects with no fibrosis compared with those with mild fibrosis – the two groups that are most difficult to differentiate using other modalities, such as elastography. An interesting study would be to evaluate A2M in conjunction with elastography to determine if the two non-invasive modalities are complementary in predicting the presence and severity of liver fibrosis.

Unique tumor-associated molecule supports the growth and progression of oral carcinoma

Evaluation of: Jin G, Kawsar H, Hirsch S *et al.*: An antimicrobial peptide regulates tumor-associated macrophage trafficking via the chemokine receptor CCR2, a model for tumorigenesis. *PLoS ONE* 5(6), 1–14 (2010).

Monocyte chemoattractant protein (MCP)-1 is the chemokine most often associated with the recruitment of macrophages to the site of a tumor. In this study, the authors demonstrate that it is not MCP-1, but rather human β -defensin (hBD)-3 that is produced by oral carcinoma *in situ* (OCIS), and that hBD-3, rather than MCP-1, correlates with the recruitment of macrophages in these lesions.

It is known that interactions between tumor cells and leukocytes can influence the development and progression of tumors. Tumor-associated macrophages

(TAMs) play a major role in this process. Previous studies indicate that TAMs promote the development of tumors, their presence indicates a poor prognosis, and there is involvement by many cytokines in the interaction between tumor cells and TAMs, most notably MCP-1. Nonetheless, MCP-1 is not present in OCIS lesions.

This study sought to determine which chemotactic factor is responsible for attracting TAMs to OCIS, how this is achieved and the effect on the tumor. First, the authors confirmed that hBD-3 was the chemoattractant responsible for attracting TAMs by comparing its expression and distribution with that of hBD-2 in normal and tumor tissue. Using co-immunofluorescence staining, they found that although both hBD-2 and -3 are found in normal and tumor tissue, hBD-2 was more prevalent in normal tissue while hBD-3 was more prevalent in tumor tissue. The authors also demonstrated that hBD-3-expressing cells had chemokine

receptor-2-expressing macrophages in close proximity to these tumor cells.

In a murine xenograft model, the authors demonstrated that human embryonic kidney 293 cells that overexpressed hBD-3 formed tumors at all sites of inoculation (compared with only half of the inoculated sites for control cells), and that tumors overexpressing hBD-3 were larger than controls. hBD-3 induced the expression of multiple interleukins and TNF- α in macrophages, derived from peripheral blood monocytes. Monocyte cell migration is mediated by chemokine receptor-2 in response to hBD-3.

These data suggest that hBD-3 uses chemokine receptor-2 to recruit TAMs in OCIS lesions and demonstrates the importance of the immune system in the development of human oral cavity cancer. A validation study is warranted, as are further investigations to evaluate the role that human β -defensins play in the development of other human malignancies.

High clusterin levels predate evidence of Alzheimer's disease

Evaluation of: Thambisetty M, Simmons A, Velayudhan L *et al.*: Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. *Arch. Gen. Psychiatry* 67(7), 739–748 (2010).

Currently, there is not a reliable biomarker to determine who will develop Alzheimer's disease (AD). The authors conducted genomic, neuroimaging and proteomic investigations on white Europeans enrolled in one of two studies who were classified, after cognitive assessment, as having AD, mild cognitive impairment or neither (healthy elderly individuals). Blood samples were collected and brain MRI and PET studies performed on the enrolled participants in discovery and validation studies.

The authors conducted discovery experiments on a set of 44 plasma samples from the first study using 2D polyacrylamide gel electrophoresis from subjects with AD, mild cognitive impairment or neither, and identified eight proteins that best correlated with hippocampal volume. A total of 51 plasma samples were collected from a similar cohort enrolled in the second study and eight proteins identified that were associated with rapid cognitive decline. The only protein common to both sample sets was clusterin.

From those findings, the authors hypothesized that clusterin may be useful as a marker for AD in normal non-demented individuals. They found that even in normal individuals, clusterin predicted greater fibrillar amyloid burden in the hippocampus. The authors did not find a correlation between clusterin mRNA in white blood cells and protein concentrations of clusterin in the plasma. They speculate that the primary source of plasma clusterin may be from organs other than blood, such as liver or brain.

Preclinical studies were also conducted in transgenic mice that overexpress amyloid precursor protein and mimic various aspects of AD, such as amyloid plaques, and cognitive and behavioral deficits. The authors found that mice with higher levels of amyloid precursor protein had higher concentrations of clusterin than controls, and that there was a close association between cortical amyloid- β burden and clusterin deposition.

While the authors acknowledge that their findings do not support the clinical use of plasma levels of clusterin protein as a standalone biomarker for AD, they suggest that clusterin is a key signature for AD disease pathology and support continued investigations to identify other proteins that may serve as biomarkers of AD.