Calretinin ELISA: A New Assay for the Detection of Mesothelioma in Blood Samples

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Background

The number of asbestos-associated mesothelioma is still increasing or remaining on a high level in many countries. In order to improve programs for early detection that are offered to workers with a previous exposure to asbestos, minimally-invasive markers are urgently needed. Currently, calretinin is one of the best available markers in immunohistochemistry for the diagnosis of mesothelioma. Goal of the study was to develop a new enzyme-linked immunosorbent assay (ELISA) for human calretinin and to evaluate its performance as a blood-based marker for the diagnosis pleural mesothelioma [1,2].

Methods

New antibodies against human calretinin were produced by immunization of a rabbit. The polyclonal antibodies were purified and a part of the batch was biotinylated to develop a two-site sandwich ELISA. Sample stability as well as the influence of the matrix (serum, heparin and EDTA plasma) and the concentration of calcium were investigated by testing the recovery rate of calretinin with spiked-in samples. Blood samples of 97 healthy controls, 35 asbestos-exposed workers and 49 mesothelioma patients were analyzed. The specificity of the antibodies was tested by immunohistochemistry with tissue sections of mesothelioma and cancer-free tissues.

Results

The range of detection of the new ELISA was between 0.1 and 9.0 ng/ml calretinin. Binding of the antibodies to calretinin is dependent on calcium ions. Therefore, calcium (5 mM $CaCl_2$) has to be supplemented when determining calretinin in EDTA plasma. The antigen is very stable and is able to survive storage at room temperature for several days and multiple freeze/thaw cycles. Median calretinin values in healthy controls, asbestos-exposed workers, and mesothelioma patients were 0.20, 0.33, and 0.87 ng/ml, respectively. Differences between all three groups were statistically significant (Fig. 1). Median concentrations of calretinin were similar in patients with epithelioid and biphasic mesothelioma (0.89 and 0.82, resp.; p = 0.23), while the single sarcomatoid case showed a lower concentration. Using the new antibodies, immunohistochemistry revealed a clear staining of epithelioid as well as sarcomatoid cells in tissue sections of mesothelioma (Fig. 2).

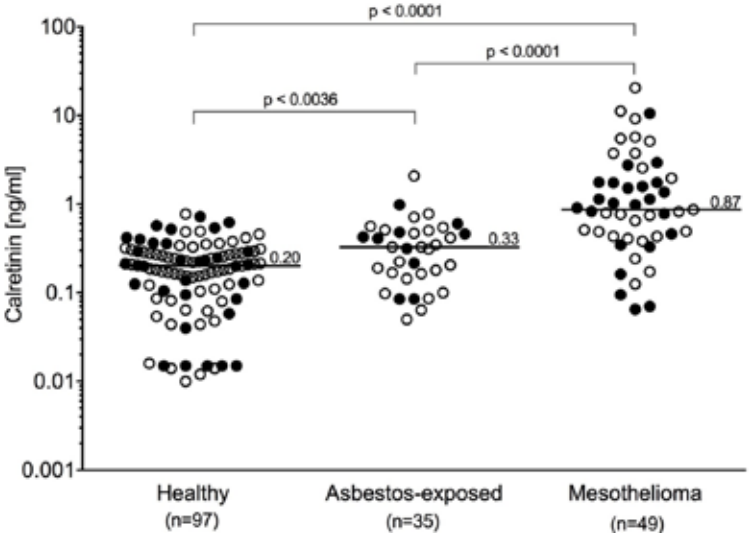

Fig. 1:

Calretinin concentrations in 97 healthy controls, 35 asbestos-exposed workers, and 49 mesothelioma patients. Differences were highly significant (p < 0.0001) between mesothelioma cases and healthy controls as well as between mesothelioma cases and asbestos-exposed workers. Within each group the differences between serum (o) and plasma (•) were not statistically significant.

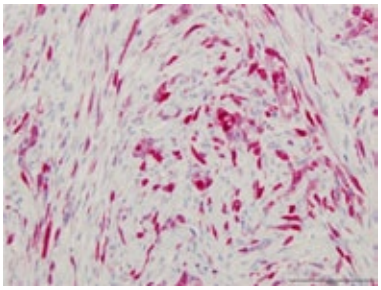

Fig. 2:

Immunohistochemical staining of a predominantly sarcomatoid mesothelioma. The new antibodies for calretinin are staining epithelioid as well as sarcomatoid cells.

Conclusions

Calretinin is a promising biomarker that can be detected in blood samples of epithelioid and biphasic mesothelioma cases. The new calretinin ELISA is robust, showed a good sensitivity, and can be used with serum as well as plasma samples. Therefore, the calretinin ELISA might be able to complement other blood-based markers, e.g., as part of a marker panel. However, more studies – with more cases and different control groups – are needed to evaluate the usefulness of the new assay for the diagnosis of mesothelioma. For a possible application in early detection of mesothelioma we are currently validating the calretinin ELISA using serial samples from a prospective cohort, consisting of former asbestos workers with asbestosis.

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