POSTER SESSION 3

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Plakoglobin immune analysis as a diagnostic test for arrhythmogenic cardiomyopathy: a comparison of immunoperoxidase and immunofluorescence methods in heart and endomyocardial biopsy samples

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Background: Arrhythmogenic cardiomyopathy (AC) is an inherited heart muscle disorder characterized by a progressive fibro-fatty replacement of cardiomyocytes, accounting for life-threatening arrhythmias and sudden cardiac death. In vivo AC diagnosis is a challenge particularly in concealed forms and the recent proposed immune-analysis test of plakoglobin (JUP) remains controversial.

Methods: Heart specimens (HS, either autopsy or transplants) and endomyocardial biopsy (EMB) formalin-fixed myocardial tissue available from 60 AC unrelated patients (42 HS and 18 EMB) and 58 non AC matched-subjects (42 HS and 16 EMB) were evaluated both by conventional immunoperoxidase analysis (IHC) and immunofluorescence (IF) using two different JUP antibody (Ab) dilutions (IHC: 1:50,000 and 1:250,000; IF: 1:1,000 and 1:50,000). Analysis was performed in areas of structurally normal myocardium. Reduced JUP signal level was defined as 50% reduction in distribution and/or intensity of the immunostained areas compared to the control samples. To exclude time-dependent tissue decay, control staining for N-Cadherin was performed. Clinical and histological data supporting diagnosis were correlated to the data of immune analysis in order to calculate sensitivity (Se) and specificity (Sp) values.

Results: Among HS, 29 of the 42 AC-cases and 22 of 42 not AC-cases had a reduced JUP immune signal with an Ab dilution 1:50,000 (69% Se; 47.6% Sp), whereas with an Ab dilution 5-fold higher (1:250,000) 36 of the 42 AC-cases and 28 of 42 not AC-cases exhibited reduced JUP immunoreactivity, increasing test Se to 86% and reducing Sp to 33.3%.

Among EMB samples, a JUP signal reduction was observed in 9 of the 18 AC-cases and 3 of 16 not AC-cases (50% Se; 81% Sp) with 1:50,000 Ab dilution, whereas with 1:250,000 Ab dilution in 10 of 18 AC-cases and in 4 of 16 not AC-cases JUP signal was reduced (Se 56%; Sp 69%).

IF data were similar both with 1:1,000 and 1:50,000 JUP-Ab dilution, indicating in HS a decreased JUP signal in 30 of 42 AC-cases and in 24 of 42 non AC-cases (71% Se; 43% Sp) and in EMB samples a decreased JUP signal in 5 of 18 AC-cases and in 3 of 16 non AC-cases (33.3% Se; 81% Sp).

Conclusion: Routine immuno-histochemical analysis of JUP signal is associated with low Se and limited Sp to be advocated as a diagnostic test both with IHC and IF. The absolute Se range was much higher in HS than EMB samples. Thus, EMB evaluation proved to be more reliable than HS in terms of test Sp. Interestingly, in HS specimens showed higher Se and lower Sp than EMB samples. Finally, high Ab dilutions confer higher Se but reduce test Sp.