



VASCULAR DISEASE, VASCULITIS

EX VIVO CONFOCAL LASER SCANNING MICROSCOPY: AN INNOVATIVE METHOD FOR DIRECT IMMUNOFLOUORESCENCE OF CUTANEOUS VASCULITIS

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Introduction: Ex vivo confocal laser scanning microscopy (ex vivo CLSM) offers an innovative diagnostic approach by vertical scanning of the skin samples with a resolution close to conventional histology. In addition, it enables fluorescence detection in the tissue, which has been shown to be helpful in fluorescence immunohistochemistry of melanoma, offering an alternative method to conventional fluorescence detection systems in dermatology. To our knowledge, the use of ex vivo CLSM in the diagnosis of cutaneous vasculitis has not been published yet.

Objective: We aimed to assess the applicability of ex vivo CLSM in the diagnosis of cutaneous vasculitis and compare its diagnostic accuracy with conventional DIF.

Methods: 82 sections of 49 vasculitis patients with relevant DIF findings were examined with ex vivo CLSM following the same staining protocol as conventional DIF examination using FITC-labeled anti-human antibodies. Two ex vivo CLSM specialists, one of them trained in histology and the other in direct immunofluorescence techniques, assessed the obtained ex vivo CLSM images.

Results: DIF showed immunoreactivity of vessels with IgG, IgM, IgA, C3 and Fibrinogen in 2.0%, 49.9%, 12.2%, 59.2% and 44.9% of the patients, respectively. Ex vivo CLSM detected positive vessels with the same antibodies in 2.0%, 38.8%, 8.2%, 42.9% and 36.7% of the patients, respectively. Detection rate of positive subepidermal vessels was significantly higher in DIF examination as compared to ex vivo CLSM ($p < 0.05$). Positive dermal vessels were identified in a higher number of patients using ex vivo CLSM as compared to DIF; however, the difference was not statistically significant.

Conclusions: Ex vivo CLSM could identify specific binding of the antibodies in subepidermal as well as dermal vessels and showed a comparable performance to conventional DIF in





diagnosing vasculitis. This study offers the first view on the possibilities of detecting skin-bond antibodies with the use of ex vivo CLSM.

