

4PS-17-04

**DOES A NEGATIVE DIRECT ANTIGLOBULIN TEST EXCLUDE WARM AUTOIMMUNE HEMOLYTIC ANEMIA?
A PROSPECTIVE STUDY OF 504 CASES**

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Introduction: Serological evidence of warm-type AIHA is generally obtained by a direct antiglobulin test (DAT). With few exceptions, a positive DAT is indicative for the presence of autoantibodies: However, DAT-negative AIHA is suspected to constitute up to 5% of all warm-type AIHAs. It can occur by several possible mechanisms, including sensitization by a small amount of IgG that falls below the detection threshold, and both IgA and IgM antibodies that remain undetected by polyspecific antiglobulin reagents (Garratty, 1993). A highly sensitive gel technique has been introduced to overcome these problems (Nathalang et al, 1997). Accordingly, a negative DAT is now often interpreted as absence of autoantibodies and thus, as more or less preclusive for the diagnosis of AIHA.

Study design: We conducted a prospective study enrolling in- and out-patients in whom immune-mediated RBC destruction was to be excluded (n=1388). An indirect antiglobulin test (IAT) and DAT including anti-IgG, -IgM, -IgA, -C3d, and 'C3c specificities, respectively, were performed using gel technique. Acid elutions were subsequently performed on RBCs from all patients who were non-reactive in IAT and DAT (n=504).

Results: Out of the 504 eluates performed, 481 were non-reactive, but 23 (4.6 %) contained RBC-specific antibodies. Two groups of patients were identified: patients with previously undiagnosed AIHA (15/23), and patients with allospecific antibodies (5/23). All AIHA patients were found to be anemic, with one exception in whom a haemoglobin concentration at the lower border of the normal range was observed. A secondary cause of AIHA was determined in 6 patients. One year after the initial diagnosis, 5 patients had responded to steroid treatment, and 7 displayed at least one positive DAT upon subsequent examinations (data on treatment not available).

Conclusions: This, to our knowledge, is the first prospective study demonstrating that even with an improved, monospecific DAT, employing gel technique, a small, but not negligible amount of AIHA patients will remain undetected. The DAT must be interpreted in conjunction with clinical and other laboratory data to avoid erroneous conclusions; it can be expected to be false-negative in up to 3% of AIHA patients. Elution of RBC antibodies is a valid additional procedure to clarify whether autoantibodies are present in DAT negative patients.