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Autoantibodies are associated with negative interference in cardiac troponin I assays.

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INTRODUCTION

Recently non-identified interfering factor (IF), which negatively influences quantitative measurements of cardiac troponin I (cTnI) in some human blood samples, was described in literature (Eriksson et al., Clin Chem 2003; 49: 1095-104). Authors spiked troponin complex in blood samples of healthy volunteers and signal was measured by several sandwich troponin I assays. It was found out that in some samples (about 3%) the recovery was significantly lower (up to 5% and even less) comparing with that in the buffer. It was suggested that some unknown factor forms complexes with the antigen and influences cTnI measurements. Some assays, utilising antibodies specific to the central part of cTnI molecule, were especially sensitive to the presence of IF in the sample. Our studies have corroborated results described in this article. Here we are also presenting our results revealing that such negative interference is associated with the presence of anti-troponin autoantibodies in human blood.

To select serum samples with elevated levels of IF, we used an IF-sensitive cTnI immunoassay described by Eriksson, which utilizes two Mabs specific to the mid-fragment of the cTnI molecule (Fig. 1).

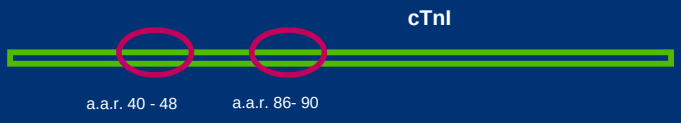

Figure 1: Epitope location of antibodies used in sandwich immunoassay, sensitive to the presence of interfering factor.

By size exclusion chromatography studies of serum samples with high IF content we demonstrated that IF activity in human blood is associated with the 100-200 kDa macromolecular fraction. It was supposed that the IF activity belongs to autoantibodies specific to the different components of troponin complex and that this autoantibody pool competes with assay Mabs for the binding sites on the antigen surface. To prove this hypothesis we loaded several individual serum samples with high IF content (recovery 6 - 20%) onto the column with Protein A Sepharose (Pharmacia, Sweden), measuring recovery in IgG depleted serum and in the protein fraction eluted from Protein A. When troponin complex (HyTest, Finland) was spiked in immunoglobulin depleted serum, the response was 90 - 110% from what we obtained with troponin spiked in Buffer A (20 mM TrisHCl pH 7.5; 150 mM KCl, 5 mM $CaCl_2$). In immunoglobulin containing fractions the recovery was significantly lower and was comparable with the recovery in the original serum sample (10 - 30%) (Fig. 2).

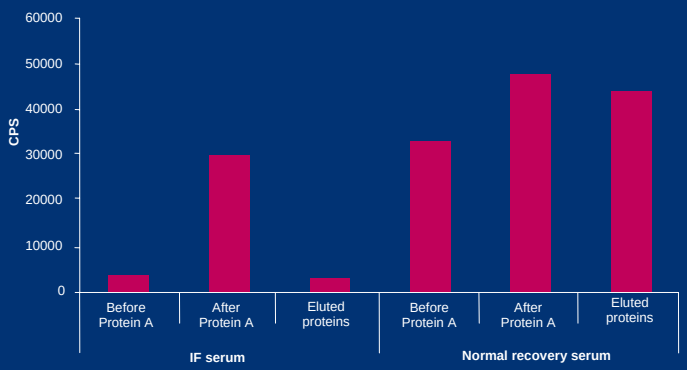

Figure 2: **Recovery in two types of serum samples (high and low recovery) before and after IgG extraction by Protein A chromatography.** Troponin complex was spiked in IF - containing and normal recovery blood samples before and after Protein A chromatography and signal was measured by the assay, sensitive to the presence of IF**.**

To extract proteins responsible for the negative interference, we prepared an affinity matrix containing immobilized human cardiac troponin complex with subunits cross-linked by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysulfosuccinimide. The serum sample exhibiting a high IF content (7.7% recovery) was loaded onto a column with the affinity matrix and the recovery was measured in the effluent and in the protein fraction eluted by 0.1 M citrate buffer, pH 3.0. We observed significant improvement of the effluent recovery (92%). The proteins eluted from the affinity matrix were separated by gel electrophoresis, blotted to nitrocellulose and stained by anti-human IgG/IgM rabbit polyclonal antibodies conjugated to HRP (Fig. 3). The immunostaining data demonstrated that two main protein bands (nitrocellulose membrane staining with Ponceau S) are represented by heavy and light chains of human immunoglobulins. In the experiment, when a normal-recovery (low IF content) serum was loaded onto an affinity column, the amount of eluted immunoglobulins was significantly lower. The recovery in the effluent slightly (104%) improved comparing with the original sample.

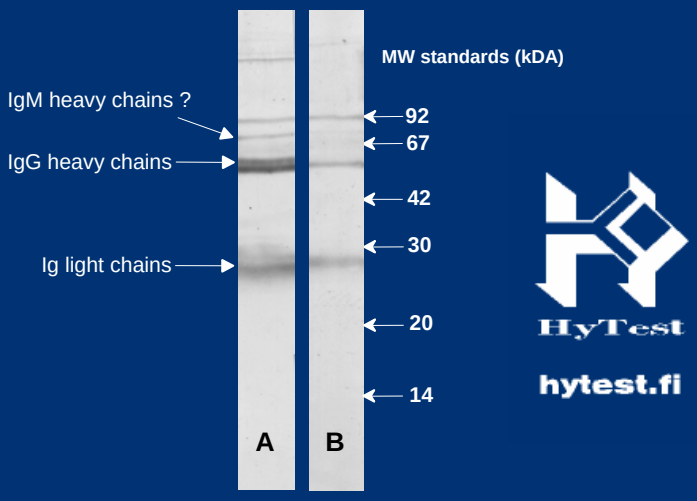

Figure 3: Serum protein fractions eluted from affinity matrix (Sepharose CL-4B with covalently bound cross-linked human cardiac troponin complex), subjected to gel electrophoresis, Western blotting and stained by anti-human IgG/IgM polyclonal antibodies conjugated with HRP. A. Serum with low recovery. B. Serum with high recovery.

The data obtained, confirm our hypothesis according to which anti-cardiac troponin autoantibodies could be responsible for negative interference in certain cTnI assays. The presence of such autoantibodies in the majority of high-IF sera was also established in our titration studies using the troponin complex or purified troponin subunits (cTnI and cTnT) as antigens (data not shown).