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Heterophilic antibody interference in a tryptase immunoassay

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Abstract

Objective: Investigation of the susceptibility of a tryptase immunoassay to interference by heterophilic antibodies.

Methods: The effect of preincubation with a blocking agent was investigated on the levels of tryptase, human anti-mouse antibodies and IgM rheumatoid factor in sera with elevated IgM rheumatoid factor levels.

Results: In 5 of 30 sera with IgM rheumatoid factor, tryptase levels were reduced at least twofold after pre-incubation with blocking reagent. A significant association was observed between the presence of IgM rheumatoid factor in the sera and the interference of tryptase immunoassay. There was no quantitative correlation found between the reduction in serum tryptase level by treatment with a blocking agent, and the amount of IgM rheumatoid factor was present. However, this reduction in serum tryptase was significantly correlated with the amount of human anti-mouse antibodies in the sera. After incubation with blocking agent, there was no change in IgM Rheumatoid factor level, but a significant decrease in human anti-mouse antibodies.

Conclusion: The Phadia tryptase assay method, in its present form, is sensitive to interference by heterophilic antibodies.

Keywords: Mastocytosis; Tryptase; Immunoassay; Interference; Rheumatoid factor

Introduction

Measurement of serum tryptase levels is an important test to perform in patients with suspected systemic mastocytosis [1]. A total tryptase level >20 ng/mL suggests underlying systemic mastocytosis [1]. An increased serum tryptase, however, is not only found in patients with systemic mastocytosis. Patients with myeloid non-mast cell lineage neoplasms, such as acute or chronic myeloid leukemias, myelodysplastic syndromes, myeloproliferative disorders or myelomastocytic leukemia, might also have elevated serum tryptase levels. The serum tryptase level might also be transiently increased during a severe allergic reaction [1]. The major criterion for systemic mastocytosis is the identification of a compact, dense, multifocal infiltrate of mast cells in a bone marrow biopsy section or other internal organ [1]. Therefore, a recently observed elevated tryptase level result in adults with suspected mastocytosis is most often followed by a bone marrow biopsy.

Recently we described a patient with a falsely-elevated tryptase level, attributed to heterophilic antibodies, which led to an unnecessary bone marrow biopsy [2]. As this was the first description of a falsely-elevated tryptase assay result, it was decided to further investigate the susceptibility of this tryptase assay method to interference by heterophilic antibodies. Sera with elevated rheumatoid factor activity were selected, because heterophilic antibodies often exhibit properties of autoantibodies and frequently are IgM rheumatoid factor [3,4]. We investigated the effect of preincubation with a blocking agent, referred to as the HBT heterophilic blocking tubes, on the levels of tryptase, human anti-mouse antibodies (HAMA) and IgM rheumatoid factor in sera.

Methods

Sera were selected from left-over samples previously submitted for routine diagnostic combined analysis of IgM rheumatoid factor and anti-cyclic citrullinated peptide antibodies.

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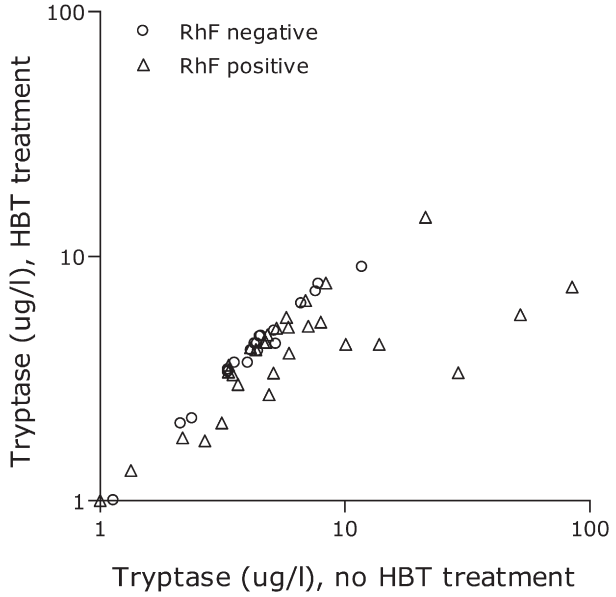

Fig. 1. Measurement of tryptase before and after incubation of serum with blocking agent (HBT).

IgM rheumatoid factor was determined as described previously, using aggregated rabbit IgG as antigen [5]. Tryptase in serum was determined with the UniCAP tryptase fluor-enzyme-immunoassay using the UniCAP 100 instrument, according to the manufacturers' instructions (Phadia, Nieuwegein, Netherlands). The 'Directions for Use' of this tryptase assay procedure reports 11.4 μg/L as the 95% upper percentile value for healthy children and adults. A 3% within-assay coefficient of variation at this tryptase level is reported by the manufacturer. Heterophilic Blocking Tubes (HBT) were obtained from Scantibodies Laboratory, Inc, Santee, CA, USA. For the analysis of immunoassay interference, sera were incubated for one hour in HBT at room temperature, according to the manufacturers' instructions (www.scantibodies.com). Human anti-mouse antibodies (HAMA) were determined by ELISA. The HAMA ELISA kit was obtained from Roche (Almere, Netherlands). In this assay method, HAMA simultaneously binds to the biotin-labeled immobilizing mouse IgG and to the peroxidase-conjugated detection mouse IgG. This complex binds to the streptavidin-coated surface of a microtiter plate. The limit of detection of this ELISA assay is 1.5 μg/L.

Results

Of the left-over samples submitted for routine IgM rheumatoid factor analysis, 20 sera with a negative result and 30 sera with a positive result in this assay method, evenly distributed over the range 2–6400 IE/mL, were selected. The result of the tryptase measurements before and after HBT treatment is shown in Fig. 1. Untreated and HBT treated sera were both analyzed simultaneously in the same tryptase assay. Four untreated sera had a tryptase level >20 μg/L, HBT-treatment reduced this level about 10-fold in three of these sera and about one-third in the fourth serum. In two other sera with tryptase levels of 10.1 and 13.8 μg/L, HBT treatment reduced these levels more than 2-fold. The case reports of these 6 patients with HBT-responsive sera do not mention mastocytosis, hematological malignancies, anaphylaxis or symptoms of allergy. The final diagnosis of these 6 patients was viral hepatitis, rheumatoid arthritis, Cushings disease, erosive rheumatoid arthritis, active rheumatoid arthritis and rheumatoid arthritis.

We considered 12% (four times the within assay coefficient of variation of 3%) as a significant difference between two measurements in the same tryptase assay. There was a significant association between an HBT-effect of >12% and both the presence of HAMA (chi-square test, $p=0.002$) (Table 1a) and IgM Rheumatoid factor (chi-square test, $p=0.015$) (Table 1b). Considering only the 16 HAMA-containing sera with a HBT effect >12%, there was a significant correlation between the magnitude of the HBT effect and the amount of HAMA ($r_s=0.63$; $p=0.009$). No significant correlation was found between the magnitude of the HBT effect and the content of IgM rheumatoid factor ($r_s=0.17$; $p=0.519$, $N=16$).

A significant association was observed between the presence of the IgM rheumatoid factor and the presence of HAMA, chi-square test, $p=0.013$ (Table 2). However, if only the 21 sera were considered with both detectable HAMA and IgM rheumatoid factor, no significant correlation between the quantitative results of these tests was found ($r_s=0.07$, $p=0.78$).

Assigning a HAMA level of 1.5 μg/L (the detection limit of the HAMA ELISA) to sera with a HAMA level below this limit, the average HAMA level of the 30 sera with a positive result in the IgM rheumatoid factor assay was 32.3 μg/L, significantly higher (Mann–Whitney *U*-test, $p<0.008$) than the average HAMA level of the 20 sera with a negative result in the IgM rheumatoid factor assay (5.2 μg/L).

Table 1a
HAMA and HBT-induced tryptase decrease

	Tryptase decrease <12%	Tryptase decrease >12%
HAMA neg	20	3
HAMA pos	11	16

$p=0.002$.

Table 1b
IgM rheumatoid factor and HBT-induced tryptase decrease

	Tryptase decrease <12%	Tryptase decrease >12%
IgM RhF neg	17	3
IgM RhF pos	14	16

$p=0.015$.

Table 2
HAMA and IgM rheumatoid factor

	IgM RhF neg	IgM RhF pos
HAMA pos	6	21
HAMA neg	14	9

$p=0.013$.