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Protein interference in thyroid assays: an in vitro study with in vivo consequences

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Abstract

Background: Pathological concentration of plasma proteins may cause problems in immunoanalytics. The low triiodotyronine (T3) and thyroxine (T4) levels, frequently found in seriously ill patients, may be ascribed either to laboratory artifact due to the lower thyroid hormone binding capacity or to a compensatory response of the organism to the disease.

Methods: The authors performed an in vitro experiment, in which sera of seriously ill patients with either low immunoglobulin G (IgG), and/or low albumin levels were investigated for free thyroid hormones (fT3, fT4) following stepwise adjustment of the serum IgG and/or albumin. All two hormones were measured with two different automated immunoassays: the microparticle enzyme immunoassay (MEIA) with two steps (AxSym, Abbott, USA) and the electrochemiluminescence immunoassay (ECLIA).

Results: The bias of fT3 and fT4 exhibited positive correlations with serum IgG and albumin. The bias of fT3 was more pronounced than that of fT4 following the addition of albumin (40–150% and 10–40%, respectively) as well as following the addition of IgG (8–30% and 0–8%, respectively). The MEIA method was more sensitively affected in case of fT4, whereas the bias of fT3 was more influenced in the ECLIA assay. In MEIA assay, the influence of albumin on the bias of fT3 and fT4 was stronger if serum IgG levels were low.

Abbreviations: MEIA, microparticle enzyme immunoassay; ECLIA, electrochemiluminescence immunoassay; NTI, non-thyroid illness; FFA, free fat acid.

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Conclusion: The results confirm that pathological thyroid findings in seriously ill patients may largely be ascribed to some laboratory artifacts.

Keywords: IgG; Albumin; Free thyroid hormones; Immunoassay; Bias

1. Introduction

The effect of pathological concentrations of normal plasma proteins is a well-known phenomenon in immunoanalytics [1,2]. Certain amount of bovine serum albumin is necessary in the incubation buffer for the formation of the antigen–antibody complex and to reduce nonspecific binding. In the case of the free hormone estimations, the addition of exogenous proteins was thought to make the assay robust against nonesterified free fat acids (FFAs), and to counteract the negative bias caused by dilution [1–4]. For the reproducibility and precision of the immunoassays, it is essential that the protein concentration of the calibrating standards should not be significantly different from the in vivo situations. In the case of analytes bound with high affinity to carrier proteins (e.g. steroids, free thyroid hormones), the matrix effect can be extremely disturbing. This is one of the reasons why some investigators interpreted the low thyroxine (T4) and even more so the low triiodotyronine (T3) levels in 'non-thyroid illness' (NTI) as possible laboratory artifacts [3,5,6]. Other investigators, on the other hand, view NTI as a self-standing pathophysiological entity [6–8]. One aim of the current study is to provide further experimental evidence with regard to this problem.

Several investigators [2,5,7,9] studied the influence of thyroxin binding globulin (TBG), together with albumin, as a possible cause of the matrix effect in thyroid assays. There is much less evidence [10] for the role of immunoglobulins and especially for immunoglobulin G (IgG) and albumin together. In the current study, a series of in vitro experiments were performed, in which the sera of severely ill patients with low IgG and/or low albumin concentrations were selected. Exogenous IgG or albumin was added separately, stepwise to these sera. Concentrations of free T4 (fT4) and free T3 (fT3) were measured with two routinely used, however, principally different automated immunoassay procedures: the two-step microparticle enzyme immunoassay (MEIA) and the electrochemiluminescence immunoassay (ECLIA) with antibody labeled competition principle. A further aim of the experiment was to analyze the possible interference caused by wide ranges of serum IgG and albumin concentrations on free thyroid hormones.

2. Materials and methods

2.1. Patient sera

Aliquots of blood sera (surplus volumes left from the routine investigations) of 33 severely ill patients (23 males and 10 females with a mean age of 51±17 years) of the Intensive Care Unit and the Department of Medicine were used for the study. Patients suffered from nephrosis syndrome (N=13), liver cirrhosis (N=12) or common variable or some other humoral immunodeficiency syndromes (N=8). Following the measurement of the IgG and albumin concentrations (for estimations see below), sera were individually assigned into three groups: a group of normal albumin and low IgG, low albumin and normal IgG or low albumin and low IgG. Sera were kept refrigerated until measurements (at −20 °C, up to no longer than 2 months). Relevant biochemical parameters of the three patient groups are summarized in Table 1. The study was performed in compliance with the Helsinki Declaration of 1975 as revised for human studies in 2000.

2.2. In vitro adjustment of serum protein levels

2.2.1. IgG adjustment

The initial IgG concentration of the pooled serum was 3.5±0.6 g/L. Exogenous IgG (5% solution of