Clinical Biochemistry 51 (2018) 72–79

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Review

Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences

Christopher R. McCudden[a,*], Joannes F.M. Jacobs[b], David Keren[c], Hélène Caillon[d], Thomas Dejoie[d], Kristian Andersen[e]

[a] Dept. of Pathology & Lab. Medicine, Division of Biochemistry, University of Ottawa, Canada
[b] Department of Laboratory Medicine, Radboud University Medical Center, Laboratory Medical Immunology, Nijmegen, The Netherlands
[c] Division of Clinical Pathology, Department of Pathology, The University of Michigan Hospital and Health Systems, United States
[d] Laboratoire de Biochimie, Institut de Biologie, Hôtel Dieu, CHU de Nantes, France
[e] Department of Hematology, Odense University Hospital, Odense, Denmark

ARTICLE INFO

Keywords:
Serum protein electrophoresis
Interferences
Monoclonal therapy
IgG4
Antibiotics
Contrast dye
Fibrinogen
Hemolysis

ABSTRACT

Protein electrophoresis and immunofixation are subject to a variety of analytical interferences that may affect monoclonal protein diagnostics performed in the context of monoclonal gammopathies. Interferences include endogenous substances, such as hemoglobin and fibrinogen, and exogenous compounds, such as radiocontrast dyes, antibiotics, and monoclonal antibody therapies. General approaches to managing interferences begin with recognition of the problem. Provided herein are examples of common, rare, and novel interferences with the goal of providing a comprehensive overview. With each example, specific methods and strategies are provided to manage analytical interferences to ensure that interpretative reports are accurate. Longstanding and newer technologies are also described to contextualize where interferences may be identified and avoided.

1. Introduction

Analytical interferences are important causes of laboratory error [1,2]. It is important for laboratories to recognize analytical interferences as they may have clinically significant consequences, including over or under treatment and misdiagnosis. Analytical interferences affect most available technologies, from enzyme assays to chromatography. Serum protein electrophoresis (SPE) and immunofixation (IFE) are no exception, where both agarose gel and capillary-based methods are subjected to an array of interferences. Interferences may derive from both endogenous substances that occur naturally, or resulting from pathophysiology and exogenous compounds in the form of medical therapies.

Endogenous interferences that may affect SPE/IFE testing include hemolysis, fibrinogen, and rare antibodies, whereas exogenous compounds include radiocontrast dyes, antibiotics, and more recently, monoclonal therapies. These interferences affect different technologies variably, with consequences ranging from a false positive screening test, in the case of hemolysis mimicking an abnormal band with SPE, to polyclonal IgG4, which may mimic a monoclonal gammopathy. While some interferences, such as fibrinogen, are readily removed once recognized, others, such as monoclonal therapies are much more difficult to manage. Irrespective of the source and nature of the interference, it is essential that reporting laboratories, those who interpret the results, and those that act on the results are aware of potential interferences.

Laboratories and interpreters must be uniquely adept at identifying and, where possible, eliminating interferences in the interest of providing accurate results. The objective of this review is to provide a comprehensive overview of the different sources and mechanisms of interference with different technologies and strategies to cope with them.

2. Endogenous interferences

2.1. Fibrinogen

The glycoprotein fibrinogen is the substrate for thrombin and is cleaved into fibrin to form the fibrin clot in the final step of the coagulation cascade. In case of adequate pre-analysis, fibrinogen is not normally present in serum specimens. However, fibrinogen may be present in serum of patients with disorders of coagulation, or those patients receiving anticoagulation therapy. It may also be encountered when a plasma sample is erroneously provided instead of serum. When SPE is performed on these samples, fibrinogen migrates to the β/γ-

* Corresponding author.
E-mail address: cmccudde@uottawa.ca (C.R. McCudden).

http://dx.doi.org/10.1016/j.clinbiochem.2017.08.013
Received 9 August 2017; Received in revised form 21 August 2017; Accepted 21 August 2017
Available online 24 August 2017
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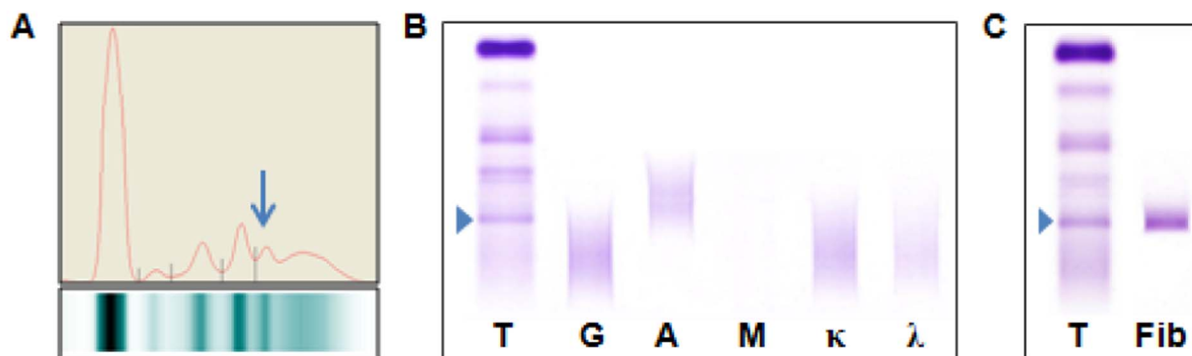

Fig. 1. Protein electrophoresis of serum sample with fibrinogen. A) Additional band of approximately 3 g/L (indicated by blue arrow) is observed with SPE that is located in the β/γ-fraction of the spectrum. B) IFE with anti-γ, α, μ, κ and λ sera illustrates that the focal band in the β/γ-region is not a monoclonal immunoglobulin. C) IFE with an antibody against fibrinogen provides proof that the band is caused by fibrinogen interference. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

region and it may be misinterpreted as a monoclonal immunoglobulin (Fig. 1A). The absence of the apparent monoclonal protein following immunofixation electrophoresis (Fig. 1B), combined with the characteristic localization of the band in the β/γ-region, should establish the identity of this band as fibrinogen. In diagnostic practice, timely recognition of this phenomenon is important to prevent that the band in SPE is misclassified as a monoclonal protein (M-protein). Although not routinely performed in diagnostic practice, IFE with anti-fibrinogen antibodies provides solid proof that the band is indeed fibrinogen (Fig. 1C).

Snyder et al. have reported a case in which IFE revealed an apparent α heavy-chain that was caused by precipitation of fibrinogen with IgA antiserum. This finding could be explained by the fact that the IgA antiserum used for IFE cross-reacted with fibrinogen [3]. For proper M-protein analysis of these rare cases, either another blood sample should be obtained or it is recommended to selectively eliminate the fibrinogen prior to protein electrophoresis [4].

As a general recommendation, laboratory specialists that interpret SPE should be aware of potential (fibrinogen) artifacts, and they should use IFE to confirm apparent SPE abnormalities. Use of IFE for confirmation testing is useful for detection of other types of artifacts and should be used widely to confirm any abnormality noted by SPE or urine protein electrophoresis (UPE).

2.2. Hemolysis

Hemolysis is a commonly encountered interference with many laboratory tests. Hemolysis refers to rupture of red blood cells causing release of cytoplasmic contents into serum or plasma and can affect test methods in several ways. The two main interference mechanisms are spectral interference from high concentrations of hemoglobin and direct release of analytes from red blood cells. Red blood cells contain relatively high concentrations of hemoglobin, potassium, magnesium, iron, phosphate, lactate dehydrogenase, and aspartate aminotransferase. Thus, any degree of hemolysis artifactually elevates these analytes in serum and plasma. Spectral interference is caused by the high concentration of hemoglobin released during hemolysis and its effect on absorbance readings. Hemolysis is readily recognized either visually, or preferably using serum indices, which automatically flag samples with hemolysis; automated detection of hemolysis provides more consistent results than individual visual inspection [5,6].

Hemolysis can be broadly divided into either pre-phlebotomy (in vivo hemolysis) or during phlebotomy (in vitro) causes. Causes of in vivo hemolysis are numerous and include microbiological agents, pre-eclampsia, hemolytic anemia, and inborn errors, such as sickle cell disease. In vitro causes usually result from mechanical rupturing through incorrect needle sizes (shear force), excessive needle suction, or prolonged storage [7]. In addition, there are conditions which make red blood cells more fragile, such as increased membrane rigidity in neonates [8], increased red blood cell fragility in elderly hospitalized patients, and those undergoing chemotherapy may be susceptible to drug-induced autoimmune hemolytic anemia [9].

Irrespective of the cause of hemolysis, release of red cell cytoplasmic contents affects serum protein electrophoresis directly. In SPE, hemoglobin and hemoglobin-complexes show up as discrete bands in the alpha-2 and beta regions (Fig. 2). These additional bands may be misinterpreted as monoclonal proteins by SPE testing. This is easily avoided by identifying hemolyzed specimens prior to interpretation and/or by reflexing to IFE to confirm the presence of any abnormal band. In general, hemolysis is not severe enough to prevent identifying monoclonal gammopathies (i.e. sample re-collection is not necessary), but there is the quantitative issue where alpha-2 and beta fractions are falsely elevated by hemolysis. Where laboratories choose to quantitate monoclonal proteins in the alpha-2 or beta regions, there is a greater risk for error in reporting falsely elevated values due to the presence of hemoglobin complexes.

As with so many interferences, awareness of the phenomenon, its

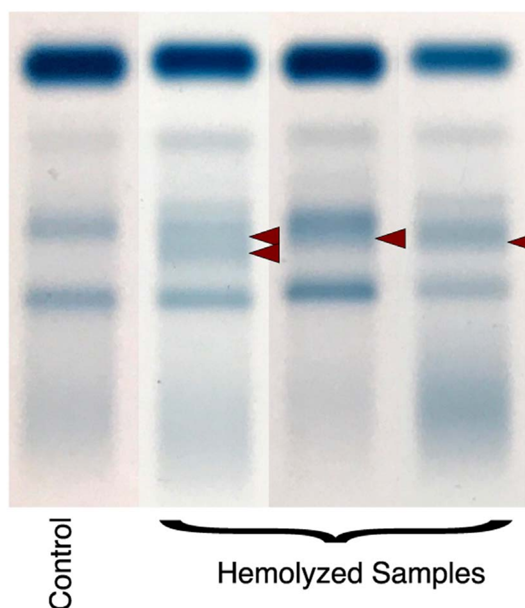

Fig. 2. Effect of hemolysis on SPE. Red arrows denote hemoglobin-haptoglobin complexes caused by gross hemolysis. Hemolysis typically increases the concentration of the alpha-2 and/or beta regions depending on the type of gel used (split beta vs. single beta). Such complexes are also evident by capillary electrophoresis. Immunofixation shows no evidence of a monoclonal protein. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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