Editorial

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Biotin interference on immunoassay methods: sporadic cases or hidden epidemic?

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At present time, immunoassay systems, using fully automated platform, are the methods of choice in the clinical laboratory, especially for the measurement of particularly complex heterogeneous molecules, which are present in biological fluids in concentration in the range of ng/L or less [1–3]. It is not surprising, as immunoassays involve the reaction of complex biological reagents (like antibodies) with other complex biological reagents (like some peptides and proteins) in a variable biological matrix, that they are inherently vulnerable to different types of interference. Unfortunately, the frequency with which such interference may occur – and most importantly, the proportion of consequently erroneous results that may significantly and adversely affect clinical management – is rather difficult to assess [2, 3].

It is well known for a long time that drugs can often affect the laboratory test results by interfering with the analytical systems themselves, or by influencing endogenous constituents [4]. Indeed, clinicians are accurately informed, and so they are usually aware on the effects of pharmacological treatment on laboratory test results, like as those of amiodarone on metabolism of thyroid hormones [5], or those of diuretics on plasma electrolytes and volume homeostasis [6], as well as those caused by toxic effects of chemotherapy on several tissues [7]. However, drug interferences often go unrecognized in the laboratory due to lack of relevant patient medication information [8].

The interferences of immunoassay methods affecting the analytical systems themselves are less known by the clinicians, and so they are more clinically insidious. Among these interferences, those altering antibody binding are probably the most frequent, especially the interferences due to heterophile antibodies and human anti-mouse antibodies (HAMA), depending upon the assay and the analyte [1]. Other interferences, affecting the formation of antigen-antibody complex, can be related to streptavidin-biotin interaction.

The streptavidin-biotin interaction provides an efficient and convenient method for accurately separating free from bound antigen in both competitive and noncompetitive immunoassay methods [9–11]. Indeed, the high-affinity interaction between streptavidin and biotin is not disturbed by multiple washings, and biotinylation typically does not alter biological activity or immunologic specificity when bound to a molecule [9]. This methodology is currently and widely used in several immunoassay systems using fully automated platforms, including: Access, DxI and DxC (Beckman Coulter); Elecsys, Cobas and Modular platforms (Roche Diagnostics); Isys platforms (Immuno Diagnostic System); Ortho Vitros platform (Ortho Clinical Diagnostics); Dimension Vista, Exl, Immulite platforms (Siemens Healthineers) [11]. However, it is important to note that biotin-streptavidin technology is used only by some (but not all) the immunoassay methods, supported by these fully automated platforms [11]. Accordingly, the biotin interference should be evaluated specifically for each immunoassay methods. Some recent studies suggested that the results of immunoassay methods, using streptavidin-biotin interaction methodology, can be affected by the presence of anti-streptavidin antibodies [10] or very high biotin circulating levels due to supplemental therapy [11–13]. However, the evidences so far reporting about biotin interference on immunoassay methods are prevalently based on case reports, rather than on experimental or clinical studies, as recently reviewed in detail [11].

In this issue of the *Journal*, Piketty et al. [14] evaluated the assay interference on several immunoassays for thyroid, steroid and protein hormones (i.e. FT3, FT4, PTH, TSH, 25OH-vitamin D, cortisol, FSH, LH, PTH, and C-peptide), using Cobas e411 platform (Roche Diagnostics), in subjects receiving moderate to very high doses of biotin. The aim of this study was to evaluate the relationship between the daily biotin dose, the plasma biotin concentration, and the magnitude of analytical errors. The degree of interference of biotin concentration was estimated before and after adsorption of biotin, present in the sample, to magnetic microparticles coated with streptavidin, and also by measuring the same plasma samples with other two immunoassays, not based on the biotin-streptavidin procedure for the separation of free from bound antigen (i.e. Liaison XL platform by Diasorin for FT4 and PTH

assays, and Access platform by Beckman Coulter for TSH assay) [14]. The biotin concentrations ranged from 31.7 to 1160 ng/mL in 23 plasma samples of patients with multiple sclerosis or voluntary subjects supplemented with daily dose up to 300 mg of biotin [14]. After the removal of biotin by adsorption, the vitamin concentration fall below the detection limit of immunoassay in all but two samples. Most hormonal results using the Cobas e411 platform were abnormal in subjects/patient supplemented with biotin, and normalized after vitamin adsorption with magnetic microparticles coated with streptavidin, suggesting a significant presence of interference. On the contrary, all results of alternative methods were normal except two slight PTH elevations with Liaison platform. Furthermore, the percentage change in hormone concentrations before and after adsorption procedure correlated strongly with the biotin concentration before the procedure, strongly suggesting the biotin is responsible of this interference. The results of this study [14] demonstrate that immunoassays, based on the biotin-streptavidin procedure for the separation of free from bound antigen, can be significantly affected by high biotin concentrations, leading to a high risk of misdiagnosis.

Biotin supplementation has progressively expanded over the last years, due to both medically prescribed therapies and vitamin complex preparations purchased for personal dietary supplements [11]. Recently, high doses of biotin were fond to be a therapeutic option in biotin responsive basal ganglia disease, an orphan neuro-metabolic disease caused by mutation in the *SLC19A2* gene coding for a thiamine transporter [15]. Moreover, high doses of biotin, ranging from 100 mg to 300 mg per day, which are 10,000 times the daily recommended intake in general population, are being investigated as a treatment for progressive multiple sclerosis [16]. As far as dietary supplementations are considered, the number of subjects involved, and especially the doses taken by subjects/patients are unknown. Indeed, because biotin is included in several very popular poly-vitamin complex preparations, individuals are not aware to take this vitamin and biotin intake is often not reported in medical history. Consequently, detection of interference from biotin ingestion requires early suspicion through collaboration between laboratory and clinical staff and an understanding of local prescribing (and patient self-prescribing) practice.

Piketty et al. [14] reported that susceptibility of immunoassays to the biotin interference was highly variable: PTH assay was least affected, while 25OH-vitamin D assay was most affected. Clinically misleading results were observed for 25OH-vitamin D and PTH (biotin concentration ≥ 169 ng/mL), FSH, LH and TSH (≥ 180 ng/mL), fT4 (≥ 233 ng/mL), fT3 and cortisol (≥ 363 ng/mL), and C-peptide (≥ 487 ng/mL). Considering these results, biotin shows a significant interference (>10%) when plasma concentrations is >30 ng/mL, and the concentrations measured in healthy subjects without vitamin supplementation is <5 ng/mL (which is also the limit of detection of the liquid chromatography-mass spectrometry procedure used by the Authors) [14]. Piketty et al. [14] reported that healthy volunteers taken 15–30 mg/daily dose of biotin can actually show concentrations >30 ng/mL, and so should present some significant interferences in immunoassays, based on biotin-streptavidin technology, and using several fully automated platforms by Roche Diagnostics.

Piketty et al. [14] should be praised not only for demonstrating that biotin can significantly interfere some popular immunoassay methods for peptide, steroid and thyroid hormones, but also for reporting a specific procedure able to confirm that biotin itself is responsible of this interference. It is important to note that another group independently reported a similar procedure to test the presence of biotin interference in clinical samples, based on the adsorption of biotin with magnetic microparticles coated with streptavidin [17]. The results of these two studies [14, 17] demonstrate that the adsorption of biotin with magnetic microparticles coated with streptavidin should be considered to be a simple and accurate laboratory test to evaluate the biotin interference in clinical samples. Of course, the results of these studies [14, 17] should be confirmed for other automated platforms using immunoassays, based on biotin-streptavidin technology for separation of bound to free antigen.

From a clinical point of view, it is important that immunoassay methods other than hormones should be tested for biotin interference, in particular those for cardiac biomarkers. It is conceivable that highly sensitive immunoassays for cardiac troponins, using biotin-streptavidin technology, should be vey sensitive to this type of interference, due to very low concentrations of biomarker, which should be measured. Furthermore, it should be considered from a clinical point of view that even very low interference at the level of decision cut-off value (i.e. 99th percentile of reference population) may induce misdiagnosis.

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