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Case Report

An increased CDT camouflaged a monoclonal light chain gammopathy: An approach for diagnosis

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ABSTRACT

Introduction: Carbohydrate-deficient transferrin (CDT) is the most reliable indicator for the detection of chronic alcohol consumption. Recently, we have investigated a clinical case in which a concomitant monoclonal light chain gammopathy mimicked an increase of this biomarker.

Materials and methods: A patient's serum was routinely examined by capillary electrophoresis (CE) for evaluation of CDT, and it was subsequently analysed through high-performance liquid chromatography (HPLC) to confirm the referred result. Then, according to the patient's clinical history, we performed serum and urine immunofixation, together with k and λ free light chain measurement.

Results: The pathological CDT value obtained by CE agreed with the patient's previous data, but it was not confirmed by the HPLC. The patient's medical record revealed hypogammaglobulinaemia since 2006, which had been recently examined by a haematological visit. Serum and urine immunofixation revealed a light chain gammopathy, which had been suspected but never confirmed by laboratory assessment. The k and λ free light chain measurement completed the diagnostic process.

Conclusion: To the best of the authors' knowledge, this is the first study of its kind to report on a perfect camouflaging of a monoclonal light chain as disialo-transferrin. The importance of the careful examination of the patient's clinical history for the correct evaluation of laboratory results, thereby preventing misinterpretations, is also highlighted.

1. Introduction

Carbohydrate-deficient transferrin (CDT) emerged as the most reliable indicator for the detection of chronic alcohol consumption [1], as highlighted in the recommendations of the Working Group on Standardization of CDT measurement (IFCC-WG-CDT) [2]. CDT can detect a group of minor isoforms of human transferrin with a lower degree of glycosylation than the major isoform of this glycoprotein [3].

Another biomarker is ethyl glucuronide (EtG), a metabolite derived from nonoxidative alcohol metabolism, which can be evaluated in the hair to detect the presence of chronic abusers, according to the recommendations of the Society of Hair Testing (SoHT) [4].

We have recently dealt with the case of a suspended airplane pilot in our laboratory. The reason of the suspension was found to be an increased CDT value, which was confirmed by two tests run in two different laboratories, with the same methodology, that is, capillary electrophoresis (CE). However, this was not confirmed through EtG on the hair analysis. We investigated this clinical case by applying both the methods routinely used for CDT detection, and we extensively investigated the protein pattern by considering the patient's medical record, according to the guidelines for the investigation of monoclonal gammopathies [5].

2. Materials and methods

CDT was determined with CE on the Sebia Capillarys System (Sebia) and the Chromsystems CDT reagent kit (Chromsystems) on high-performance liquid chromatography (HPLC, Kontron Instruments). Serum and urine immunofixation was performed using the Sebia agarose gel System (Sebia). Latex FLC k and λ reagent kit on BN II (Siemens) was used to determine k and λ free light chains.

All the instruments and kits were used in compliance with the manufactures' instructions.

2.1. Patient clinical history

Table 1 summarizes the laboratory results. The following is a brief report of the patient's clinical course.

In May 2006, hypogammaglobulinaemia was observed by routine laboratory examinations. In December 2014, after a routine health assessment that confirmed the decrease of gamma globulins, a haematological evaluation was suggested. At the same time, the patient was suspended from active service to investigate the haematological condition. In

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Table 1
Summary for the patient's laboratory results. MCV: mean cellular volume; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase.

Date	MCV	AST	ALT	GGT	CDT-CE	CDT-HPLC	Total Proteins	Gamma %	Gamma	κ	λ	κ free	λ free	κ/λ
	(84–90 fL)	(5–35 U/L)	(6–59 U/L)	(11–68 U/L)	(<1,3%)	(<1,8%)	(g/L)	(11.10–18.80%)	(g/L)	(6.29–13.50 g/L)	(3.13–7.2 g/L)	(6.7–22.4 mg/L)	(8.3–27,0 mg/L)	
05/05/2006	86.6	26	46	32			6.5	9.1	0.59					
12/05/2006	88.0						7.6	8.4	0.64					
01/06/2007	88.3	28	45	42			7.1	10.7	0.76					
02/11/2010	86.3	15	15	24										
14/12/2011	88.8	20	23	30										
10/12/2012	86.5	19	27	43										
09/12/2013	85.1	22	22	24										
11/12/2014	88.1	17	22				6.8	7.2	0.49					
30/01/2015							6.5	7.1	0.46	3.84	3.07			1.25
11/02/2015	86.4	21	26	31			6.7	7.7	0.52					
22/05/2015							7.2	7.5	0.54					
03/06/2015	87.3	20	28	22	3.00			7.6						
06/06/2015										3.83	2.48			1.54
25/06/2015					2.80									
02/07/2015														
12/10/2015	87.7	22	26	33	2.90			7.2						
22/10/2015	91.0	16	25	31	2.60	0.91	6.8	7.1	0.48			5.2	380	0.01

March 2015, the haematologist requested the free κ and λ light chain evaluation and the Bence Jones protein determination. The subsequent serum electrophoresis confirmed hypogammaglobulinaemia. The κ and λ light chains were analysed as total chains (not as free chains as requested) and both were found to be below reference range. The κ/λ ratio was unbalanced, but this information was not considered. The CDT analysis reported a pathological result.

The second haemato-oncologic visit took place in June 2015, with the diagnosis of hypogammaglobulinaemia. The pilot was authorized to resume flight active service. The clinician suggested a control visit within 1 year. No treatment was suggested or started, because of the absence of monoclonal gammopathy diagnosis. Despite the authorization for the haematological condition, a second CDT evaluation confirmed positivity to the test (2.8%), and this stopped the rehabilitation for flight. A second level analysis for chronic alcohol consumption evaluation was suggested. The EtG on the hair of the proximal 3.5 cm provided a negative result (<15 ng/mg; positive >30 ng/mg), which contrasted with the CDT value. EtG half-life included and exceeded the time frame of positive CDT results.

In October 2015, the CDT analysis (CE) resulted positive again and the serum electrophoresis confirmed a marked hypogammaglobulinaemia. As a consequence, the suspension period was extended and further investigations were requested.

3. Results

The patient visited our laboratory in November 2015. In compliance with our diagnostic procedure for CDT evaluation, we performed the first-level analysis with CE, which confirmed the positivity (2.6%) (Fig. 1a). We then carried out the second-level analysis with HPLC, which reported a negative result, CDT 0.91% (positive >1.8%) (Fig. 1b). Furthermore, the clinical history revealed that the patient had consulted a haematologist for hypogammaglobulinaemia. We therefore decided to measure k and λ free light chains in serum and we analysed the patient's urine to investigate the presence of Bence Jones protein (Fig. 1d). The serum immunofixation (Fig. 1c) showed the presence of free λ light chains and confirmed the presence of the monoclonal λ light chains in the β1 region. These were responsible for the interference in the CDT evaluation by CE.

4. Discussion

The evaluation of alcohol abuse, despite the consolidated method for its determination, is vulnerable to some unexpected setbacks. The difference in biomarkers; half-life (CDT 1.5 weeks; EtG 1 month/cm of hair from scalp) as well as the alcohol consumption degree is well known to obtain a positive result. In fact, it takes 2 weeks of heavy alcohol consumption (at least 50/60 alcohol g/day[6]) to obtain a CDT value that will give a positive result. These considerations are essential when interpreting results and also while reading publications such as Bianchi et al. [7], where different methods are compared without considering the biomarkers' metabolism. Furthermore, the study conducted by Bianchi et al. considered AST (aspartate aminotransferase), ALT (alanine aminotransferase), GGT (gamma-glutamyl transferase) and MCV (mean cellular volume) as classical biomarkers. These are of no value to forensic toxicology investigations; nevertheless, Bianchi et al. compared them with the CDT and EtG results.

A better view on this subject was proposed by Neels et al. [8], who highlighted the advantages of EtG in hair over CDT (without any mention on AST, ALT, GGT and MCV), in particular when considering chronical alcohol consumption and its degree of evaluation.

We performed CDT analysis with CE, but we received direct requests for HPLC analysis (*e.g.* from driver licence commission). We then processed all the samples that show interferences or artefacts on the CE electropherogram using HPLC. Veronesi et al. [6] suggested analysis by two different methods, based on different principles, to confirm positive