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Original Scientific Paper

DOES LIPEMIA AFFECT COMMON COAGULATION TESTS?

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SUMMARY – Lipemia is a common preanalytical source of errors and sample rejection, especially in photometric assays. The aim of this study was to assess the effect of lipemia on the most common coagulation tests; prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and antithrombin.

Leftover citrate plasma samples were used to prepare the plasma pool which was subsequently spiked with raising amounts of lipid solutions. All tests were performed on a BCSXP® analyser (Siemens, Erlangen, Germany) utilizing the reagents from the same manufacturer. The effect of lipemia was assessed by comparing the difference in results between the native sample, the sample with the added interferent and the reference change value (RCV). Additionally, the correctness of the reaction curve of each analyte was taken into account.

PT results were not affected by triglyceride concentrations below 26.1 mmol/L when measured at 570 nm. However, if measured at 405 nm, results were acceptable only when triglyceride concentrations were not higher than 14.4 mmol/L. For APTT results, triglyceride concentrations below 12.8 mmol/L at 405 nm and below 20.3 mmol/L for 570 nm met eligibility criteria. Fibrinogen values are acceptably accurate only when triglyceride concentrations were under 12.8 mmol/L. Finally, no clinically significant triglyceride interference was found for the antithrombin test results with triglyceride concentrations of up to 37.8 mmol/L.

Interfering substances may impact coagulation assay results differently, depending on the parameter and method reaction. In this study, the threshold of lipid interferences was established for PT, APTT and fibrinogen, as well as for antithrombin measurements, which proved to be the least affected.

Keywords: *preanalytical phase; lipemia; coagulation tests; interference; urgent laboratory*

Introduction

Preanalytical issues in hemostasis testing are an important potential cause of erroneous results which might cause diagnostic errors and significant adverse clinical events[1]. In hemostasis, even more than in other laboratory disciplines, quality is required in

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every preanalytical step — from patient preparation to blood withdrawal[2]. Clinical laboratory staff needs to stay alert in discovering potential preanalytical errors in cases of unexplained abnormal coagulation test results and should provide adequate and fast solutions to minimize the potential safety risk for the patient[1]. Prothrombin time (PT), activated partial thromboplastin time (APTT), international normalized ratio (INR), fibrinogen (FBG) and antithrombin (AT) are the most frequently performed routine coagulation tests and they are used in the assessment of bleeding or thrombotic disorders, as well as to monitor anticoagulant therapy[3]. In the coagulation laboratory utilizing optical clot detection methods, as well as in other areas of spectrophotometric laboratory testing, the presence of excessive amounts of lipids in test samples presents one of the leading preanalytical challenges. The most common cause of elevated triglycerides is inadequate blood sampling after a meal or parenteral administration of synthetic lipid emulsions. Unfortunately, in urgent medical situations, fasting is not a procedure of choice to eliminate preanalytical interferences caused by elevated triglycerides in a sample[4,5]. Coagulation assays based on mechanical or electromechanical procedures are less prone to lipemia interference. When using optical clot detection, this can be minimized by comparing the absorption of samples at two wavelengths, or performing coagulation assays at alternative wavelengths[4]. The interference observed in lipemic samples is most prominent at wavelengths lower than 500 nm and can therefore be partly prevented with readings at 570 nm or above[1]. In order to reduce the number of sample rejections and improve patient safety, each laboratory should examine and define objective criteria for a particular interference in coagulation tests. Although most modern coagulometers can determine lipemia, icterus and hemolysis automatically by using plasma indices, numerous coagulation analyzers do not have this possibility[6]. For the BCS XP® analyzer, it is worth examining the effect of lipemia on the most used coagulation tests in the emergency laboratory. In this manner, it is possible to standardize the criteria for sample rejection and to minimize them. This procedure is particularly important in the emergency department, which is a specific entity and requires rapid decision-making to provide patient care as quickly as possible. Therefore, the aim of this study was to assess the interfering effect of lipemia on the most requested coagulation tests (PT, APTT, FBG, AT) by artificially inducing lipemia.

Materials and methods

The study was performed in the emergency laboratory at the Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Croatia.

Materials

A citrated plasma pool was prepared after routine laboratory processing. All samples used in the study were leftover citrated plasma samples, otherwise destined for discarding, for which the respective tests were ordered as part of routine laboratory testing. Samples were drawn into coagulation tubes (BD Vacutainer, USA) with 0.105 M Na_3 citrate and centrifuged for 10 minutes at 4000 rpm. No additional sample was collected specifically for this study. PT, APTT, fibrinogen and antithrombin were determined in the plasma pool.

Table 1. The concentration of triglycerides in the native plasma pool sample was 2.8 mmol/L. By adding 20 uL of intralipid solution to the proper plasma pool volume, additional final triglyceride concentrations were obtained.

Final triglyceride concentration (mmol/L)	Volumen of added intralipid solution (uL)	Volumen of added plasma pool (uL)
72.8	20	180
37.8	20	380
26.1	20	580
20.3	20	780
16.8	20	980
14.4	20	1180
12.8	20	1380
11.5	20	1580
10.6	20	1780
9.8	20	1980
6.2	20	3980
2.8	0	200

At the same time, 20 μL amounts of intralipid solution (SmofKabiven® zentral, Fresenius Kabi Germany GmbH, Bad Homburg, Germany) were added to eleven aliquoted portions of the plasma pool to achieve the final concentrations of triglycerides and the same tests were performed subsequently in each spiked pool sample. All results were multiplied by a dilution factor.

Methods

The concentration of triglycerides in the native and spiked pool samples was determined by the standardized enzymatic colorometric method utilizing the Cobas c501 analyser (Roche Diagnostics GmbH, Mannheim, Germany). All samples were analyzed within four hours from blood collection.

All tests were performed on a BCS® XP analyzer, utilizing reagents from the same manufacturer (Siemens, Erlangen, Germany). The used reagents were Dade® Innovin Reagent, Dade® Actin® FS Activated PTT Reagent, Multifibren* and Berichrom ® Antithrombin III with no change in the reagents' serial numbers during this study. The BCS® XP detection method is based on optical reaction at the standard wavelength of 405 nm. The recommended wavelength for lipemic plasma samples is 570 nm, because light absorption is less influenced by triglyceride absorbance[7].

Evaluation of lipemia interference

The clinical relevance of the interferences produced by adding different amounts of lipids was estimated according to the reference change value (RCV) calculated according to the following formula:

RCV= $\sqrt{2}$ + 1,96 + $\sqrt{CVi^2 + CVa^2}$; RCV, reference change value; CVi, within-subject biological variation; CVa, analytical imprecision.

Within-subject biological variation (CVi) data for fibrinogen and prothrombin time were obtained from the Westgard site, while Moniek P. M. de Maat et al. provided data for APTT and antithrombin[8,9].

Analytical imprecision (CVa) was calculated using data from method verification conducted following the protocol recommended by the Clinical and Laboratory Standards Institute (CLSI EP15-A3)[10]. Data for CVi, CVa and RCV are shown in Table 2. In addition to RCV, result acceptability was estimated by visual inspection of each reaction curve which was compared to the one generated by analyzing the original plasma pool.

Table 2. The criteria for within-subject biological variation (CVi), analytical imprecision (CVa) and reference change value (RCV) are listed.

	CVi %	**CVa %**	**RCV %**
PT	4	4.8	15.5
APTT	7.1	6.0	25.8
FIBRINOGEN	10.7	2.4	30.4
ANTITHROMBIN	7.8	6.4	28.0

Results

Data of coagulation tests in spiked pool samples are shown in Table 3. Triglyceride concentrations in spiked samples ranged from 6.2 to 72.8 mmol/L.

Our results have shown that prothrombin time is not affected by triglyceride concentrations below 26.1 mmol/L if measured at 570 nm. However, at standard wavelengths (405 nm) results were acceptable only when triglyceride concentrations did not exceed 14.4 mmol/L. It should be noted that an interference at this wavelength was also observed with a triglyceride concentration of 11.6 mmol/L, but it was possible (although highly inappropriate for an emergency laboratory) to obtain the correct value by examining and manually reading the reaction curve. Regarding APTT, the results were dubious and interference was observed on reaction curves at both wavelengths, which rendered visual inspection of reaction curves necessary. At 405 nm, results were satisfactory only when triglyceride concentrations were below 12.8 mmol/L, while at 570 nm it was possible to obtain correct results at concentrations below 20.3 mmol/L. Fibrinogen values were shown to be acceptable at triglyceride concentrations below 12.8 mmol/L. Finally, no triglyceride interference was found with antithrombin levels of up to 37.8 mmol/L utilizing an RCV value of 28%.

Table 3. Analytical data in spiked pool samples with different concentrations of triglycerides are shown. "/" no measurement required; "-" could not be measured, "" result does not meet RCV criteria, "**" result does not meet eligibility reaction curve criteria*

Examined coagulation tests	Triglycerides (mmol/L)											
	2.8	**6.2**	**9.8**	**10.6**	**11.5**	**12.8**	**14.4**	**16.8**	**20.3**	**26.1**	**37.8**	**72.8**
PT (405 nm)	0.39	0.39	0.39	0.38	0.96 *,**	0.39	0.39	0.45 **	0.47 **	0.54 *,**	0.08 *,**	0.07 *,**
PT (570 nm)	/	/	/	0.40	0.39	0.40	0.40	0.41	0.40	0.41	0.55 *,**	0.75 *,**
PT (570 nm, manually determined)	/	/	/	/	0.39	/	/	/	/	/	/	/
APTT (405 nm) (s)	36.6	18.0 *,**	16.0 *,**	14.6 *,**	15.6 *,**	15.7 *,**	35.9 **	39.0 **	58.3 *,**	48 *,**	48 *,**	138.7 *,**
APTT (405 nm, manually determinated) (s)	/	36.8	36.6	36.6	35.8	35.4	/	/	/	/	/	/
APTT (570 nm) (s)	/	32.3	14.8 *,**	14.6 *,**	14.7 *,**	13.7 *,**	13.0 *,**	12.7 *,**	12.8 *,**	35,8 **	36,7 **	- *,**
APTT (570 nm, manually determinated) (s)	/	35.8	35.6	36.6	36.6	35.3	35.1	35.1	35.3	/	/	/
FIBRINOGEN (g/L)	5.4	4.9	6.0	6.6	5.7	5.6	6.9 *	5.7 *,**	4.0 *	- *	- *	1.3 *
FIBRINOGEN (570 nm) (g/L)	/	/	5.8	6.1	/	/	9.5 *	9.5 *	6.0 *	9.4 *	9.2 *	- *
ANTITHROMBIN (%)	89.1	79.3	75.7	86	86.6	86.2	85.6	84.8	83.8	73	78	63.5 *

Discussion

Lipemia is a well recognized cause of interference for a wide range of laboratory tests. It represents an analytical challenge irrespective of lipid origin (native or intravenous lipid preparations). The most commonly reported interferences are observed with optical methods where the mechanism of interference is caused by a scattering of light away from the original path as well as absorption of light by lipids (mainly chylomicrons and very low-density lipoproteins)[8]. Therefore, it is extremely important to know all factors that may cause clinically significant differences in results and, if possible, eliminate them in a timely manner. Numerous already published studies explore lipemia interference on different analitical platforms, mostly in the general or outpatient population[6,7,11,12]. In this paper, we report the effect of lipemia on common coagulation tests performed on the BCS® XP analytical platform in the emergency department, which is a specific entity and requires rapid decision-making, and in patients on oral anticoagulant therapy. We also explored the possibilities to report adequate results in low quality samples in situations when new sampling is not an option.

By artificially inducing lipemia, we found that each test included in the study was differently affected by lipemia and that an analysis at an alternate wavelength of 570 nm provided more accurate results with increasing lipemia, regardless of the assay. Our results are in concordance with the study by Junker et al.[7], who have also shown that the 570 nm method should be preferably used for measuring plasma samples with interefering substances. The assays most sensitive to lipemia interference were APTT and fibrinogen, yielding the lowest triglyceride concentration threshold at which coagulation curve reading was not affected by lipemia. Fibrinogen sensitivity to lipemia was also confirmed in the study by Negrini et al.[6], who further suggested

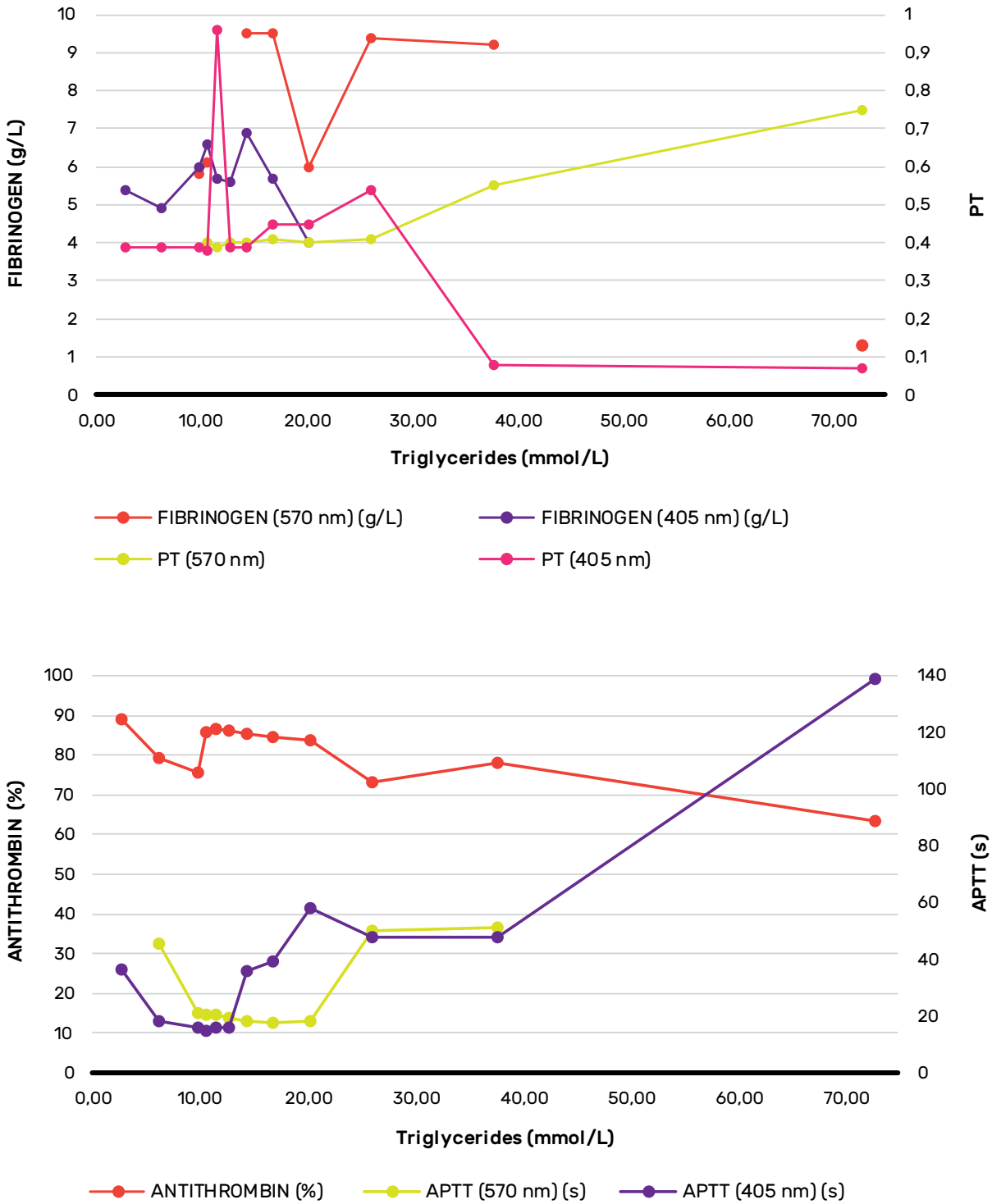

Figure 1. The impact of lipemia on results released by the analyzer for each examined coagulation test.

that this problem could be overcome by high-speed centrifugation. In our study, the lipemia thresholds represented by triglyceride concentrations that were found to affect global coagulation assays were considerably above physiological triglyceride concentrations, thus implying that reliable analysis was performed for the vast majority of patients. The results of our study confirm observations published by Woolley et al.; that each coagulation assay is differently affected by lipemia[13].

However, the case mix of patients at our emergency laboratory often includes critically ill patients on continuous parenteral nutrition with highly lipemic samples, which implies the need to set objective criteria for sample acceptance or rejection.

Our study included a wide range of triglyceride concentrations in one level of the plasma pool. The authors are also aware of the fact that triglycerides are not the only lipid particles that contribute to sample turbidity, which is a limitation in all lipemia interference studies[14]. Undoubtedly, for a more detailed understanding of the effect of triglycerides on coagulation tests, it is necessary to investigate the effect of samples with a broad range of PT, APTT, fibrinogen and antithrombin results in order to determine whether the established cut-off values of lipid interference remain the same.

Conclusions

Our study provides insight into the effect of lipemia on coagulation tests in emergency laboratories based on optical reading and sets acceptance criteria when lipemic samples are analyzed on a BCS XP® analyzer. Lipemia may impact coagulation assay results differently, depending on the parameter and method reaction. In this study, the threshold of lipid interferences was established for PT, APTT and fibrinogen, as well as for antithrombin measurement, which proved to be the least affected.

Conflicts of interest

None declared.

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Sažetak

UTJEČE LI LIPEMIJA NA NAJČEŠĆE KOAGULACIJSKE PRETRAGE?

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Lipemija je čest predanalitički izvor pogrešaka i razlog neprihvaćanja uzoraka, osobito u fotometrijskim analizama. Cilj ovog istraživanja bio je procijeniti učinak lipemije na najčešće koagulacijske pretrage: protrombinsko vrijeme (PV), aktivirano parcijalno tromboplastinsko vrijeme (APTV), fibrinogen i antitrombin.

Preostali uzorci citratne plazme korišteni su za pripremu „poola" plazme u koji su naknadno dodane rastuće količine lipidnih otopina. Sva ispitivanja provedena su na BCSXP® analizatoru (Siemens, Erlangen, Njemačka) koristeći reagense istog proizvođača. Usporedbom razlike u rezultatima između nativnog uzorka i uzorka s dodanim interferentom i vrijednosti „reference change value" (RCV) procjenjen je učinak lipemije. Dodatno, u obzir je uzimana i ispravnost reakcijske krivulje pojedinog analita.

Na rezultate PV-a nisu utjecale koncentracije triglicerida manje od 26,1 mmol/L kada su mjerene pri valnoj dujini od 570 nm. Međutim, kada se mjerenje provodilo na 405 nm, rezultati su bili prihvatljivi samo kod koncentracije triglicerida manjih od 14,4 mmol/L. Za rezultate APTV-a, koncentracije triglicerida ispod 12,8 mmol/L pri 405 nm i manje od 20,3 mmol/L pri 570 nm ispunile su kriterije prihvatljivosti. Vrijednosti fibrinogena bile su prihvatljive samo kada su koncentracije triglicerida niže od 12,8 mmol/L. Konačno, nije pronađena klinički značajna interferencija triglicerida za rezultate antitrombinskog testa s koncentracijama triglicerida do 37,8 mmol/L.

Interferirajuće tvari mogu različito utjecati na rezultate koagulacijskih pretraga, ovisno o parametru i korištenoj metodi. U ovoj studiji utvrđen je prag interferencije lipida za PV, APTV i fibrinogen, kao i za mjerenja antitrombina, kod kojih je utvrđen najmanji utjecaj interferenta.

Ključne riječi: *predanalitička faza; lipemija; koagulacijski testovi; interferencije; hitni laboratorij*