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Article

How Does Contrast Media Affect Cardiac Markers and Coagulation Tests? Experimental Study

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Abstract:

Background and objectives: The fact that the results of troponin and Nt-proBNP interfere from biotin caused some commercial firms to update their measurement methods. In particular, the clinical incompatibility of cardiac test results may affect the risk of morbidity and mortality. The aim of this study is to investigate the interference effects of 7 different contrast agents on cardiac markers (Troponin-I, Nt-proBNP, Mass CK-MB, CK, AST, LDH) and coagulation tests (PT, APTT).

Materials and Methods: Seven different contrast medias were added into control materials by using interference protocol. Concentration of PT, APTT, CK, AST, LDH, Mass CK-MB, Troponin-I, Nt-proBNP were measured by Sysmex CS-2100, Abbott c16000, Siemens Centaur XP and AFİAS-6 analyzer. The amount of deviations from target values were calculated.

Results: 7 different contrast medias caused negative interference in troponin levels between 57.43% and 62.87%. It was found that different contrast medias produced false negativity in the pro-BNP test ranging from 6.11% to 96.01%. Enzymes and coagulation tests have been less affected.

Conclusions: Different contrast medias may cause false negative cTnI and pro-BNP. Therefore, the contrast medias which causes the least interference should be preferred. The results of samples taken in the first hour of contrast imaging should be interpreted with care.

Keywords: Contrast media; Troponin; Pro-BNP; Interference

1. Introduction

Contrast media are intravenous diagnostic medias used in radiological imaging techniques. While contrast media are known to cause clinical side effects after intravenous injection, there has been less interest on the extent of their interference with laboratory results [1]. Timing of this interference appears to be unpredictable, leading to test results that are inconsistent with the clinical presentation. Such inconsistency may cause overlooking cardiac emergencies or delayed diagnosis, thereby affecting morbidity and mortality risks. Severity of interference with contrast media (iohexol, gadobutrol, gadopentetic acid, gadodiamide, ioversol) used during imaging techniques for diagnosis and follow-up purposes may differ depending on the duration of excretion from the body. In addition to several interference factors, false low cardiac marker measurements associated with biotin interference have led certain commercial companies to re-assess their measurement methods[2,3]. Any endogenous and exogenous substance found in the blood may have interference potential, and this should be considered when interpreting the results. The aim of the present study

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is to investigate the interference effect of different contrast media on cardiac markers (troponin, NT-proBNP, mass CK-MB, CK, AST, LDH) and coagulation tests (PT, APTT, fibrinogen).

2. Materials and Methods

2.1. Materials: Cardiac markers plus and system control materials (Biorad, US, Irvine, CA, Lot:23662) were used in the study. Omnipaque (iohexol, 755 mg/mL, 100 mL for intravenous injection, GE Healthcare), Emaray (gadopentetic acid dimeglumine salt, 469.01 mg/mL, 15 mL IV solution for injection), Gadotu (gadodiamide, 287 mg/mL, 15 mL IV solution for injection), Optiray (ioversol, 741 mg/mL, 100 mL for intravenous injection), Kopaq (iohexol, 755 mg/mL, 100 mL for intravenous injection), Gadovist (gadobutrol, 604.72 mg/mL, 15 mL IV solution for injection), Gadodiem (gadodiamide, 287 mg/mL, 15 mL IV solution for injection) were used as radiographic contrast media for the interference study.

2.2. Measurement Devices: ADVIA Centaur XP (Siemens Medical Solutions Diagnostics), Abbott Architect C16000 (USA), Sysmex CS2100i (Milton Keynes, UK) auto analyzer and AFIAS-6 analyzer system (Boditech Med Inc, Korea) were calibrated and utilized for measurements.

2.3. Sample Preparation: 180 microliters (uL) of control solution was placed in a centrifuge tube. Subsequently, 20 uL contrast medium was added and mixed on a vortex plate for 5 seconds. A different control solution for each test was read on analyzers. Each measurement was performed in triplicate and mean value of the 3 measurements was calculated. This measurement was performed separately for 7 different contrast media. In order to rule out the interference likely to occur due to the volume expansion with the control material, 20 uL distilled water was added, followed by a triplicate measurement and mean value of the 3 measurements was accepted as the target. Owing to the copyright of commercial companies, commercial names of the contrast media were coded from RS1 to RS7. Ethics committee approval was not deemed necessary since the study did not require any blood or tissue samples of animal or human origin.

3. Results

Deviations from the target value (bias %) were calculated in the interference study performed with 7 different contrast medias. RS6 (gadodiamide, 287 mg/mL) with 11.2% and R7 (gadodiamide, 287 mg/mL) with 10.7% were found to be the agents with the maximal effect on prothrombin time. INR deviations were similar to the findings observed for prothrombin time. The maximal effect on APTT was seen with RS2 at 11.24% (ioversol, 741 mg/mL) and RS3 at 16.47% (iohexol, 755 mg/mL). Negative interference of 0.93%-5.12% and 0.96%-4.81% was noted for CK and AST, respectively. AST was not affected by RS1 (iohexol). Negative interference of 1.66%-7.88% was seen for LDH with all contrast medias except gadobutrol (RS5). RS5 (gadobutrol) had positive interference on LDH measurement at a rate of 8.71%. RS1, RS2, RS3, RS4, RS6 influenced the mass CK-MB test at rates of 0.2% to 4.2%. Negative interference was noted for mass CK-MB at a rate of 11.37% with RS5 and 39.02% with RS7. All of the contrast medias had negative interference with troponin-I ranging from 57.43% to 62.87%. The negative interference with NT-proBNP test was found to be in the range of 6.11% to 96.01%.

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Table 1: Effect of contrast media on cardiac markers and coagulation tests. Bias: Amount of deviation from the target value. RS1-7: Radiopaque Substance [RS1 (iohexol, 755 mg/mL), RS2 (ioversol, 741 mg/mL), RS3 (iohexol, 755 mg/mL), RS4 (gadopentetic acid dimeglumine salt, 469.01 mg/mL), RS5 (gadobutrol, 604.72 mg/mL), RS6 (gadodiamide, 287 mg/mL), RS7 (gadodiamide, 287 mg/mL)].

TEST	Unit	Target Value	RS1	RS2	RS3	RS4	RS5	RS6	RS7
PT	S	12.5	13.1	13.2	13.3	13.4	12.9	13.9	13.8
BIAS(%)			4.8	5.6	6.4	7.2	3.2	**11.2**	**10.4**
INR	-	1	1.05	1.06	1.07	1.07	1.03	1.11	1.11
BIAS(%)			5	6	7	7	3	**11**	**11**
APTT	S	24.9	26.9	27.7	29	27.3	27.1	26.5	26.8
BIAS(%)			8.03	**11.24**	**16.47**	9.64	8.84	6.43	7.63
CK	IU	215	209	204	211	212	213	209	210
BIAS(%)			-2.79	-5.12	-1.86	-1.40	-0.93	-2.79	-2.33
AST	IU	104	104	99	103	103	103	103	102.00
BIAS(%)			0	-4.81	-0.96	-0.96	-0.96	-0.96	-1.92
LDH	IU	241	222	235	237	236	262	222	224
BIAS(%)			-7.88	-2.49	-1.66	-2.07	8.71	-7.88	-7.05
Mass CK-MB	ng/mL	12.775	12.35	13.3	12.5	12.3	11.3	12.775	17.725
BIAS(%)			-3.14	4.31	-1.96	-3.53	**-11.37**	0.20	**39.02**
Troponin-I	ng/mL	5.05	2.075	1.99	2.15	2.102	1.875	1.93	1.92
BIAS(%)			**-58.91**	**-60.59**	**-57.43**	**-58.38**	**-62.87**	**-61.78**	**-61.98**
Pro-BNP	pg/mL	338.73	150.73	78.79	115.81	318.05	13.53	204.36	175.07
BIAS(%)			**-55.50**	**-76.74**	**-65.81**	-6.11	**-96.01**	**-39.67**	**-48.32**

4. Discussion

Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted. Strengths and limitations of the study should be discussed as well.

Contrast medias are commonly used during radiological imaging studies. In addition to problems such as clinical side effects and drug interactions, these compounds may also lead to analytical errors in emergency laboratory tests. Accurate measurement of cardiac tests is critical in emergency care and intensive care setting owing to the lack of sufficient time to carefully assess analytical problems. In the present study, coagulation tests (PT, INR, APTT), spectrophotometric biochemistry tests (CK, AST, LDH) and tests performed by means of immunochemical methods (Troponin-I, mass CK-MB, NT-proBNP) which are commonly used particularly in cardiology units were investigated in terms of their interference with 7 different commercial contrast medias on the basis that tests exceeding a bias limit of 10% could lead to erroneous decisions at the point of clinical decision making. Positive interference with prothrombin time (PT) at rates of 11.2% and 10.4% was observed with two products (RS6, RS7) from two different commercial companies containing the same amount of gadodiamide as active ingredient. The other contrast medias (RS1-5) led to positive interference on PT ranging from 3.2% to 7.4%. Since calculation of INR level is based on prothrombin time, a similar deviation was also seen in INR values. Activated partial thromboplastin time (APTT)

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deviated by 11.24% with ioversol (RS2) and 16.47% with iohexol (RS3). The interference with other contrast medias appeared to be lower (6.43%-9.64%). Caution should be exercised for false elevated PT, INR and APTT results following imaging tests, especially those performed with gadodiamide and iodine-containing contrast media (ioversol, iohexol). Dosing based on false elevated results in situations requiring anticoagulation may lead to microaggregate formation, posing a potential risk of associated complications.

CK, AST and LDH are the biochemistry tests with limited specificity for the evaluation of cardiac functions. Enzymes were the tests exposed to least interference in the present study. Negative interference was noted for CK and AST, ranging at 0.93% to 5.12% and up to 4.81%, respectively. Negative interference of 1.66%-7.88% was seen for LDH with all contrast medias except gadobutrol (RS5). The maximum positive interference with LDH was seen with gadobutrol (RS5) at a rate of 8.71%. Due to their low specificity and the likelihood of hemolysis influence, enzymes are less valuable than troponin and mass CK-MB for clinical decision making in ACS diagnosis. However, since they are less influenced by contrast media, the diagnostic value of these tests becomes more important following contrast-enhanced imaging.

Rapid immunoassay kits for measuring mass CK-MB concentration utilize highly specific and sensitive diagnostic monoclonal antibodies [4]. However, certain immunoglobulins found in the blood may form macro-CK, leading to false positive mass CK-MB results [5]. The fact that mass CK-MB is not influenced by hemolysis makes this test more advantageous than the troponin test. Furthermore, mass CK-MB was found to be the immunoassay test with the least contrast media interference in the present study. RS1, RS2, RS3, RS4, RS6 led to deviations ranging from 0.2% to 4.31%. Negative interference of 11.37% and positive interference of 39.02% were noted for the gadobutrol-containing R5 and gadodiamide-containing RS7, respectively. The lowest rate of 0.2% interference seen with RS6 makes this agent more advantageous than RS7, which contains the same active ingredient. This finding suggests that the interference is not related to gadodiamide but related to the pharmacological excipients in RS7. The gadodiamide-containing RS7 was observed to be the contrast media with the maximum number of tests showing interference (PT, INR, mass CK-MB, troponin-I, NT-proBNP).

Ease of interference with immunoassay methods poses risks for cardiac tests. The recently popular biotin interference noted in BNP and troponin tests has urged commercial companies to re-evaluate their methods. IFCC and FDA have drawn particular attention on this interference [2,3]. The fact that the performance of these tests depends on antibody affinity facilitates interference [6]. Although immunoassay methods are standard for troponin analysis, circulating autoantibodies may result in negative interference with troponin levels [7-9]. Troponin kits from different commercial companies may show positive or negative interference with hemolyzed samples [10]. Inconsistency between the troponin result of the patient with ACS (acute coronary syndrome) diagnosis should trigger interference suspicion. Factors leading to troponin interference include fibrin clots, heterophile autoantibodies, rheumatoid factor, elevated bilirubin, lipemia, hemolysis, elevated alkaline phosphatase activity and macro-immunocomplex formation [11,12]. A study evaluating 12 different contrast agent found false positive troponin results with two different immunoassay autoanalyzers (Dade Behring; Opus Magnum and Beckman Coulter; Access) [13]. In the present study utilizing Siemens Centaur XP, negative interference ranging from 57.43% to 62.87% was seen in troponin levels with 7 different contrast media. False negative results are more hazardous than false positive results since they lead to ruling out the actual condition. Recognition of the possibility of a false negative troponin result may have vital importance in terms of the doctor's management for a patients diagnosed with ACS.

BNP and NT-proBNP are potent markers for the prognosis and risk classification in heart failure. A study by Koglin and colleagues found a significant correlation between BNP and survival score in patients with heart failure [14,15]. Therefore, false BNP results may alter the therapeutic protocol in heart failure, potentially leading to serious effects on morbidity and mortality. Janssen and colleagues have shown interference in proBNP results in the presence of an immuno-active protein with a high molecular weight. In a study utilizing the Abbott Architect device, interference

was observed to be resolved with PEG precipitation [16]. Pan Xiaohong and colleagues determined false positive BNP results with human antimouse antibodies (HAMAs) [17]. Saenger et al. demonstrated cross-reactivity with proBNP and NT-proBNP in different BNP types [18]. In the present study, different contrast medias led to false negative results in NT-proBNP tests ranging from 6.11% to 96.01%. The safest result was seen as -6.11% with the addition of RS4 (gadopentetic acid dimeglumine salt). The highest rate of false negativity was observed with RS5 (gadobutrol) at a rate of 96.01%. A BNP value of 338.73 pg/mL was measured as 13.53 pg/mL with RS5 administration, 78.79 pg/mL with RS2, and 115.81 pg/mL with RS3. Contrast agent-induced false negative NT-proBNP results following angiography may delay the diagnosis in patients diagnosed with ACS who develop cardiac failure. The false negativity rate of 96.01% with RS5 is particularly notable and poses a risk in terms of erroneous clinical decisions.

5. Conclusions

False cardiac test results may compromise patient safety. Owing to the presence of multiple interference factors, laboratory tests should not be the sole criterion for the diagnosis of cardiac diseases. Every possible effort should be made in order to prevent or identify potential causes of error during the testing process. If necessary, a contrast medium with the lowest interference potential should be preferred. The fact that contrast media may cause false negative results in cTnI and BNP analyses at considerably serious rates should be considered and results of samples obtained within an hour after contrast-enhanced imaging should be interpreted carefully.

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