

1 Article

2 How Does Contrast Media Affect Cardiac Markers 3 and Coagulation Tests? Experimental Study

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8 Abstract:

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

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

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

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

25 **Keywords:** Contrast media; Troponin; Nt-proBNP; Interference

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28 1. Introduction

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

41 is to investigate the interference effect of different contrast media on cardiac markers (troponin,
42 NT-proBNP, mass CK-MB, CK, AST, LDH) and coagulation tests (PT, APTT, fibrinogen).
43

44 2. Materials and Methods

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46 *2.1. Materials:* Cardiac markers plus and system control materials (Biorad, US, Irvine, CA, Lot:23662)
47 were used in the study. Omnipaque (iohexol, 755 mg/mL, 100 mL for intravenous injection, GE
48 Healthcare), Emaray (gadopentetic acid dimeglumine salt, 469.01 mg/mL, 15 mL IV solution for
49 injection), Gadotu (gadodiamide, 287 mg/mL, 15 mL IV solution for injection), Optiray (ioversol, 741
50 mg/mL, 100 mL for intravenous injection), Kopaq (iohexol, 755 mg/mL, 100 mL for intravenous
51 injection), Gadovist (gadobutrol, 604.72 mg/mL, 15 mL IV solution for injection), Gadodiem
52 (gadodiamide, 287 mg/mL, 15 mL IV solution for injection) were used as radiographic contrast media
53 for the interference study.
54

55 *2.2. Measurement Devices:* ADVIA Centaur XP (Siemens Medical Solutions Diagnostics), Abbott
56 Architect C16000 (USA), Sysmex CS2100i (Milton Keynes, UK) auto analyzer and AFIAS-6 analyzer
57 system (Boditech Med Inc, Korea) were calibrated and utilized for measurements.
58

59 *2.3. Sample Preparation:* 180 microliters (uL) of control solution was placed in a centrifuge tube.
60 Subsequently, 20 uL contrast medium was added and mixed on a vortex plate for 5 seconds. A
61 different control solution for each test was read on analyzers. Each measurement was performed in
62 triplicate and mean value of the 3 measurements was calculated. This measurement was performed
63 separately for 7 different contrast media. In order to rule out the interference likely to occur due to the
64 volume expansion with the control material, 20 uL distilled water was added, followed by a triplicate
65 measurement and mean value of the 3 measurements was accepted as the target. Owing to the
66 copyright of commercial companies, commercial names of the contrast media were coded from RS1 to
67 RS7. Ethics committee approval was not deemed necessary since the study did not require any blood
68 or tissue samples of animal or human origin.
69

70 3. Results

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

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

90

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
Nt-proBNP	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

91

92 4. Discussion

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

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

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

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

126 Rapid immunoassay kits for measuring mass CK-MB concentration utilize highly specific and
127 sensitive diagnostic monoclonal antibodies [4]. However, certain immunoglobulins found in the
128 blood may form macro-CK, leading to false positive mass CK-MB results [5]. The fact that mass
129 CK-MB is not influenced by hemolysis makes this test more advantageous than the troponin test.
130 Furthermore, mass CK-MB was found to be the immunoassay test with the least contrast media
131 interference in the present study. RS1, RS2, RS3, RS4, RS6 led to deviations ranging from 0.2% to
132 4.31%. Negative interference of 11.37% and positive interference of 39.02% were noted for the
133 gadobutrol-containing R5 and gadodiamide-containing RS7, respectively. The lowest rate of 0.2%
134 interference seen with RS6 makes this agent more advantageous than RS7, which contains the same
135 active ingredient. This finding suggests that the interference is not related to gadodiamide but
136 related to the pharmacological excipients in RS7. The gadodiamide-containing RS7 was observed to
137 be the contrast media with the maximum number of tests showing interference (PT, INR, mass
138 CK-MB, troponin-I, NT-proBNP).

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

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

163 was observed to be resolved with PEG precipitation [16]. Pan Xiaohong and colleagues determined
164 false positive BNP results with human antimouse antibodies (HAMAs) [17]. Saenger et al.
165 demonstrated cross-reactivity with proBNP and NT-proBNP in different BNP types [18]. In the
166 present study, different contrast medias led to false negative results in NT-proBNP tests ranging
167 from 6.11% to 96.01%. The safest result was seen as -6.11% with the addition of RS4 (gadopentetic
168 acid dimeglumine salt). The highest rate of false negativity was observed with RS5 (gadobutrol) at a
169 rate of 96.01%. A BNP value of 338.73 pg/mL was measured as 13.53 pg/mL with RS5 administration,
170 78.79 pg/mL with RS2, and 115.81 pg/mL with RS3. Contrast agent-induced false negative
171 NT-proBNP results following angiography may delay the diagnosis in patients diagnosed with ACS
172 who develop cardiac failure. The false negativity rate of 96.01% with RS5 is particularly notable and
173 poses a risk in terms of erroneous clinical decisions.

174 5. Conclusions

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

182
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185 Ataman Gönel

186
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188
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