



# Preliminary experience with the smooth muscle troponin-like protein, calponin, as a novel biomarker for diagnosing acute aortic dissection

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on behalf of the International Registry of Acute Aortic Dissection Substudy on Biomarkers (IRAD-Bio) Investigators

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## Aims

The early diagnosis of acute aortic dissection (AD) remains challenging. We sought to determine the utility of the troponin-like protein of smooth muscle, calponin, as a diagnostic biomarker of acute AD.

## Methods and results

Immunoassays against calponin (acidic, basic, and neutral isoforms) were developed and the levels were compared in a convenience sample of 59 patients with radiographically proven AD [34 males, age  $59 \pm 15$  (SD) years] vs. 158 patients suspected of having AD at presentation (116 males, age  $63 \pm 15$  years) but whose final diagnosis was not AD. Basic calponin, which is the most specific and abundant in smooth muscle, and acidic calponin, respectively, showed greater than two-fold and three-fold elevations in patients with acute AD. Diagnostic performance as determined by receiver-operating characteristics curve analysis showed that both acidic and basic calponin have the potential to detect AD in the first 24 h [respective areas under the curve (AUCs) 0.63 and 0.58], with superior performance of basic calponin (when compared with acidic) in the initial 6 h (respective AUCs 0.63 and 0.67).

## Conclusion

Circulating calponin levels were elevated in acute AD compared with controls. These biomarkers have the potential for use as an early diagnostic biomarker for acute AD.

## Keywords

Aortic dissection • Biomarker

## Introduction

Acute aortic dissection (AD) is potentially catastrophic.<sup>1,2</sup> With high mortality and morbidity, early diagnosis is a prerequisite for improved treatment and survival.<sup>3</sup> Recent advancements in the understanding of the disease have not alleviated the challenges faced by clinicians looking for an early diagnosis.<sup>4</sup>

Biochemical diagnosis has a great potential to aid in the early diagnosis, and we have therefore developed biochemical markers

with this aim. The detection of release of smooth muscle proteins, myosin heavy chain, and creatine kinase BB-isozyme from the damaged aorta was shown to be useful within the initial 6–12 h.<sup>5–10</sup> Another marker that can extend the time-window to later stages would thus be helpful.

Given the widely accepted role of cardiac troponin as being the single most reliable biomarker of myocardial ischaemia/infarction,<sup>11,12</sup> we next pursued the diagnostic possibility of an assay of a troponin counterpart of smooth muscle, calponin, in the

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**Table 1** Normal reference ranges for the assays

	<b>n</b>	<b>Average</b>	<b>Median</b>	<b>SD</b>	<b>95th percentile</b>	<b>99th percentile</b>
Acidic calponin (ng/mL)	218	1.19	1.01	0.92	2.04	5.73
Basic calponin (ng/mL)	282	44.09	31.74	41.81	124.31	162.15
Neutral calponin (ng/mL)	230	12.61	5.00	64.72	14.08	52.65

present study.<sup>13</sup> Calponin has three isoforms—acidic, basic and neutral; basic calponin (also called h1) is the most abundant and specific isoform in smooth muscle, whereas acidic and neutral calponin (also called h2) are not thought to be specific to smooth muscle (i.e. found in both smooth muscle and non-smooth muscle tissue) and are less abundant than basic calponin in smooth muscle tissue.<sup>14</sup> We describe our preliminary experience with calponin as a diagnostic biomarker of acute AD.

## Methods

### Development of calponin assays

Monoclonal antibodies against full-length recombinant acidic calponin, peptide fragments of basic calponin (peptides included amino acids 274–281 and 289–297 of basic calponin), and full-length recombinant neutral calponin were derived, and sandwich-type enzyme immunoassays were generated according to the standard procedures and protocols.

The dynamic range for acidic calponin was 0.03–50 ng/mL, basic calponin was 4.5–2500 ng/mL, and for neutral calponin was 5–700 ng/mL. Cross-reactivity was defined when 50 ng/mL of sample was tested with 50 ng/mL of other sample added for acidic calponin was 8% against basic calponin and 0% against neutral calponin, and for basic calponin was 0% against acidic calponin and 0% against neutral calponin. Precision of the acidic calponin assay showed a coefficient of variance of 11% at 2.2 ng/mL and 11% at 140 ng/mL for the basic calponin assay. Note that cross-reactivity and precision were not determined for the neutral calponin because this assay was not further pursued after initial analysis demonstrated a lack of correlation with AD.

The normal reference ranges are shown in *Table 1*. The normal range as defined by the 95th percentile was 2.04 ng/mL for acidic calponin, 124.31 ng/mL for basic calponin, and 14.08 ng/mL for neutral calponin.

### Initial clinical studies in patients with acute aortic dissection

Fourteen centres in the USA, Europe, and Japan participated in the present study (see Appendix for a complete list of the participating centres). Most were university-based tertiary centres or affiliated centres where institutional review board approval was obtained. Consenting patients with suspicion of acute AD were enrolled. The suspicion of AD had to be high enough to order an imaging test for diagnosis. Confirmation of diagnosis was made by an imaging study. Clinical data forms were completed for each of the patients with parameters as developed by the International Registry of Acute Aortic Dissection.<sup>2</sup> Blood plasma was drawn on admission and used for measurements in the present study.

**Table 2** Patient demographics of the study population

Acute aortic dissection	59 cases (34 males, 59 ± 14.5 years)
Dissection type (Stanford classification)	
Type A	43 cases
Type B	15 cases
Type undetermined in one case	
Presentation time from onset	
<6 h	16 cases (type A 14 cases, type B 2 cases)
6–12 h	20 cases (type A 16 cases, type B 4 cases)
12–24 h	22 cases (type A 13 cases, type B 9 cases)
Non-aortic dissection	158 cases (116 males, 63 ± 14.8 years)
Final diagnosis	
Myocardial infarction	37 cases
Angina pectoris	34 cases
Pulmonary embolism	3 cases
Thoracic aortic aneurysm	17 cases
Uncertain but not aortic dissection	67 cases
Presentation time from onset	
<6 h	52 cases
6–12 h	34 cases
12–24 h	72 cases

## Results

### Patient demographics

Two hundred and seventeen patients were enrolled in the study including 59 cases of radiographically proven acute AD and 158 ‘controls’ with an initial suspicion of AD but a different final diagnosis as shown in *Table 2*. Of the 59 AD cases (59 ± 14.5 years of age), 34 were males (58%). The non-AD cases (63 ± 14.8 years of age) included 116 males (73%). Final diagnoses for the control cohort included myocardial infarction ( $n = 37$ ), angina pectoris ( $n = 34$ ), pulmonary embolism ( $n = 3$ ), non-dissecting thoracic aortic aneurysm ( $n = 17$ ) or uncertain but not AD ( $n = 67$ ). Thus, the non-AD controls who had been initially enrolled with suspicion of acute AD had coronary heart disease in 45%, non-dissecting aortic aneurysm in 11%, and pulmonary embolism in 2%.

### Circulating calponin levels in patients with acute aortic dissection

Calponin levels in the enrolled patients according to time from onset (0–6, 6–12, 12–24 h) and type of dissection (all, type A, type B) are shown in *Figure 1*. Comparison of the dynamic range of elevations in the levels in patients as defined by the 75th percentile vs. the 95th percentile of the normal range indicated that acidic calponin showed a greater than two-fold increase for all dissections presenting within the first 6 h of symptom onset (4.10 ng/mL,  $n = 16$ ; normal reference, 2.04 ng/mL) which was particularly notable for type A (4.70 ng/mL,  $n = 14$ ) when compared with type B patients (2.84 ng/mL,  $n = 2$ ). Type A patients in the 6–12 h range also showed elevations (5.08 ng/mL,  $n = 16$ ) but not type B patients (2.43 ng/mL,  $n = 4$ ). Levels began to drop-off in the 12–24 h range for type A patients (3.23 ng/mL,  $n = 13$ ) and were not significantly elevated in type B patients (2.64 ng/mL,  $n = 9$ ). Patients without AD did not show elevations at any of the examined time points (0–6 h, 2.29 ng/mL,  $n = 52$ ; 6–12 h, 2.65 ng/mL,  $n = 34$ ; 12–24 h, 2.62 ng/mL,  $n = 72$ ).

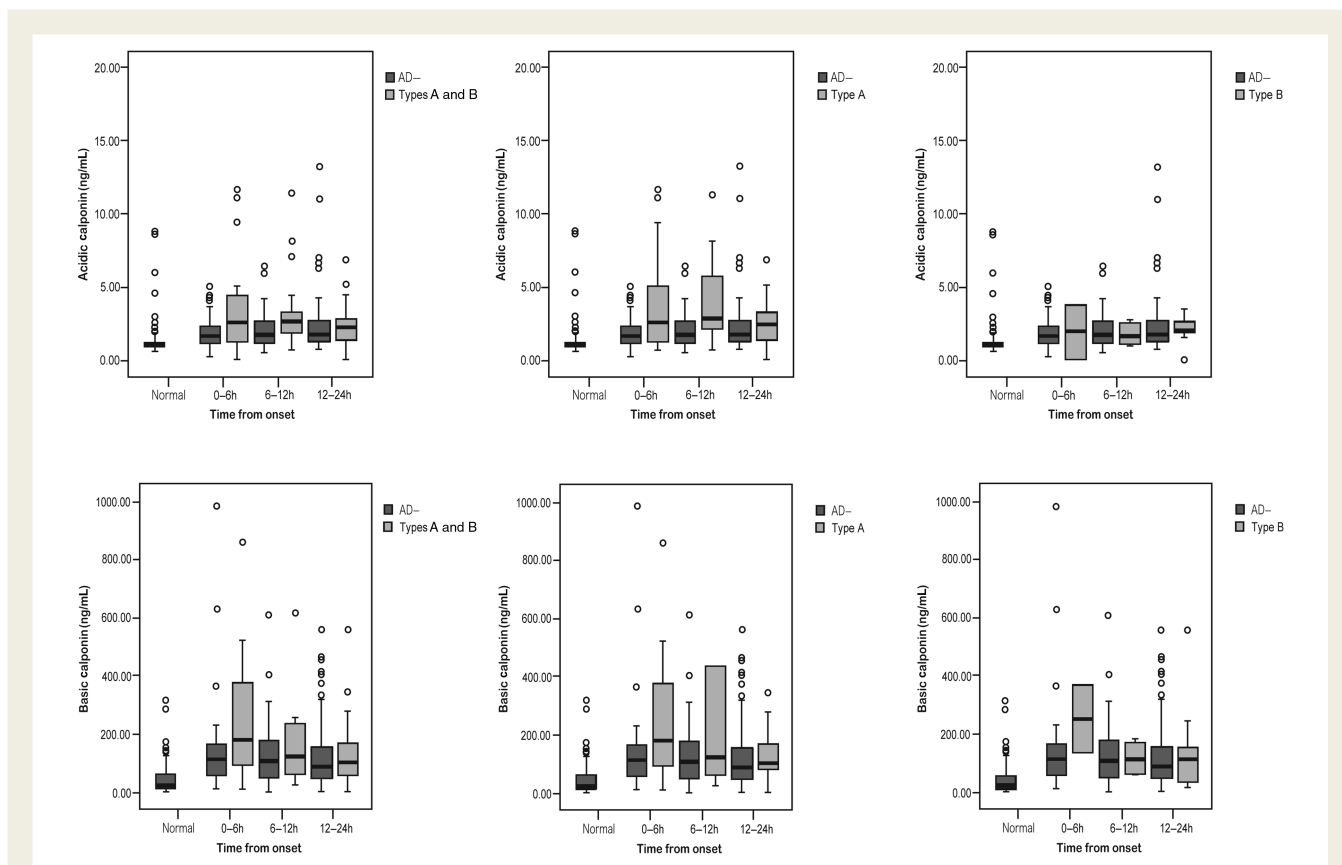
Basic calponin showed a more than three-fold increase at 377.56 ng/mL (normal reference, 123.31 ng/mL) for all dissections when sampled within the first 6 h of symptom onset ( $n = 16$ ) which was similar for type A (379.04 ng/mL,  $n = 14$ ) and type B patients (316.24 ng/mL,  $n = 2$ ). The 6–12 h time-window

showed similar, greater than three-fold, elevations in type A patients (348.79 ng/mL,  $n = 16$ ) but with a drop-off for type B patients (171.96 ng/mL). Levels in both type A and type B patients had fallen in the later 12–24 h group (all patients, 169.24 ng/mL,  $n = 22$ ; type A patients, 172.05 ng/mL,  $n = 13$ ; type B patients, 171.96 ng/mL,  $n = 9$ ). Patients without AD did not show elevations at any of the examined time points (0–6 h, 166.70 ng/mL,  $n = 52$ ; 6–12 h, 179.41 ng/mL,  $n = 34$ ; 12–24 h, 159.98 ng/mL,  $n = 72$ ).

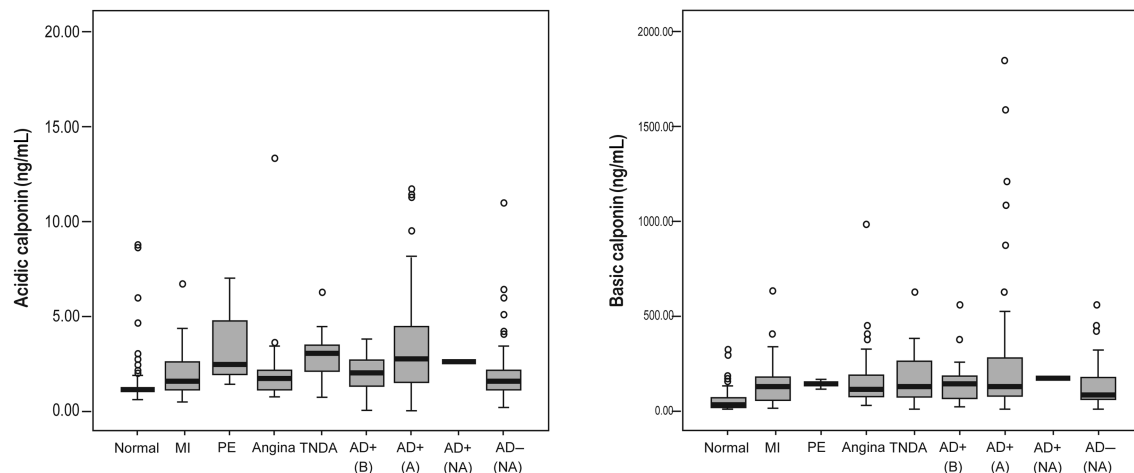
Neutral calponin did not show elevations in any AD patient regardless of type or time from onset of symptoms (0–6 h, 5.11 ng/mL,  $n = 16$ ; 6–12 h, 18.17 ng/mL,  $n = 21$ ; 12–24 h, 13.19 ng/mL,  $n = 22$ ; normal reference, 14.08 ng/mL). As expected, neutral calponin did not show elevations in non-AD controls (0–6 h, 15.03 ng/mL,  $n = 52$ ; 6–12 h, 8.19 ng/mL,  $n = 34$ ; 12–24 h, 12.30 ng/mL,  $n = 72$ ).

Thus, acidic and basic calponins showed greater than two-fold and three-fold elevations, respectively, during the initial 6 h with type A and remained elevated through to 12 h. For type B dissection, acidic and basic calponin levels were elevated in the very early presenters (0–6 h) but not afterward. Neutral calponin did not show disease-associated changes and was not further pursued.

Further analysis according to final diagnosis was done for acidic and basic calponin. As shown in *Figure 2*, AD clearly showed elevations with a marked increase in the type A patient for basic calponin.



**Figure 1** Box plots of acidic and basic calponin in patients with aortic dissection according to type (all, type A, type B) and time after symptom onset (0–6, 6–12, 12–24 h).



**Figure 2** Acidic and basic calponin levels in patients examined in the present study according to final diagnosis. MI, myocardial infarction; PE, pulmonary embolism; TNDA, thoracic non-dissecting aneurysm; AD+(B), type B aortic dissection; AD+(A), type A aortic dissection; AD(-), aortic dissection type not determined; AD(-), uncertain final diagnosis but not aortic dissection.

## Diagnostic performance of calponin assays

Sensitivity and specificity of detection of acute AD were also analysed by receiver-operating characteristics (ROC) curves. The area under the curve (AUC) values as a collective parameter reflective of diagnostic accuracy is shown in *Table 3*. Overall, for all of the patients examined within the initial 24 h period, acidic calponin showed highest AUC values at 0.63 followed by basic calponin at 0.58. Higher values were seen for type A patients alone at 0.65 and 0.59, respectively. For the early samples alone (0–6 h), basic calponin showed highest AUC values (0.67) followed by acidic calponin (0.63). Type A showed similar AUC values of 0.65 for either acidic or basic calponin. Type B showed an AUC of 0.82 for basic calponin but the sample size ( $n = 2$ ) limits the confidence of this observation.

The optimal clinical decision limit value was determined from these ROC curve analyses which showed that the optimal value for acidic calponin was 2.8 ng/mL which resulted in a sensitivity of 50% and specificity of 87% for the initial 6 h and 2.3 ng/mL which resulted in a sensitivity of 58% and specificity of 72% for the initial 24 h period. Similarly, the optimal value for basic calponin was 159 ng/mL which resulted in a sensitivity of 63% and specificity of 73% for the initial 6 h and 139 ng/mL which resulted in a sensitivity of 50% and specificity of 66% for the initial 24 h period. According to type, type A showed an optimal value for acidic calponin at 2.8 ng/mL which resulted in a sensitivity of 50% and specificity of 87% for the initial 6 h and 2.3 ng/mL which resulted in a sensitivity of 58% and specificity of 72% for the initial 24 h period. Similarly, the optimal value for basic calponin was 159 ng/mL which resulted in a sensitivity of 64% and specificity of 73% for the initial 6 h and 141 ng/mL which resulted in a sensitivity of 50% and specificity of 67% for the initial 24 h period.

The predictive values (negative and positive) as calculated with a prevalence of 1 in 10 000 were 0.84 and 0.56 in the initial 6 h and

0.84 and 0.41 in the initial 24 h, respectively, for acidic calponin and 0.86 and 0.44 in the initial 6 h and 0.80 and 0.33 in the initial 24 h, respectively, for basic calponin (*Table 3*). Importantly, both acidic and basic calponin had higher negative predictive values.

## Discussion

### Biochemical diagnosis of acute aortic dissection by circulating calponin levels

We have developed immunoassays against a troponin counterpart of smooth muscle, calponin, and have examined the circulating levels in acute AD and a number of non-dissection patients. As expected, the immunoassay developed against basic calponin, which is the most specific and abundant isoform in smooth muscle, showed the greatest elevations. Acidic calponin also showed diagnostic elevations, but disease-associated changes in neutral calponin levels were non-diagnostic. Analysis by type and time after onset showed that acidic calponin detects acute AD within the first 12 h with superior performance in type A patients. Basic calponin showed superior performance for the first 6 h. Importantly, calponin measurements allowed for detection of the disease in patients with a more delayed presentation (out to 12 h) which should be a welcome addition for diagnostic use in comparison with smooth muscle myosin heavy chain which previously has been shown to possess superior accuracy for patients presenting within 6 h after onset.

One of the strengths of the present study was that diagnostic performance of the assays was determined in patients who were enrolled on the basis of a clinical suspicion of acute AD and not by comparison with healthy controls. Thus, the test's accuracy reflects the 'real-world' conditions. We do note that many of the participating centres were major aortic centres (e.g. tertiary centres) and thus the diagnostic approach may not accurately

**Table 3** Diagnostic performance of the assays

	AD				Type A				Type B			
	Number of patients	Number of non-patients	ROC AUC	P-value	Number of patients	Number of non-patients	ROC AUC	P-value	Number of patients	Number of non-patients	ROC AUC	P-value
Early samples: AD (0–6 h) vs. non-AD (0–6 h)												
Acidic calponin <sup>a</sup>	16	52	0.63	0.11	14	52	0.66	0.07	2	52	0.46	0.85
Basic calponin <sup>b</sup>	16	52	0.67	0.04	14	52	0.65	0.10	2	52	0.82	0.13
Neutral calponin	16	52	0.42	0.33	14	52	0.39	0.20	2	52	0.64	0.49
All samples AD: (0–24 h) vs. non-AD (0–24 h)												
Acidic calponin <sup>c</sup>	59	158	0.63	0.00	43	158	0.65	0.00	15	158	0.54	0.57
Basic calponin <sup>d</sup>	59	158	0.58	0.08	43	158	0.59	0.08	15	158	0.54	0.63
Neutral calponin	59	158	0.50	0.99	43	158	0.49	0.77	15	158	0.56	0.48

NPV, negative predictive value; PPV, positive predictive value.

<sup>a</sup>NPV: 0.84; PPV: 0.56.

<sup>b</sup>NPV: 0.86; PPV: 0.44.

<sup>c</sup>NPV: 0.84; PPV: 0.41.

<sup>d</sup>NPV: 0.80; PPV: 0.33.

reflect the use in the community setting. Further, although we have examined a large number of patients of this rather uncommon disease, the analysis according to type and time after onset resulted in smaller subgroups which limited the number of patients in each subset. This limits our ability to study the diagnostic accuracy in certain cohorts (e.g. high AUC values in type B dissection for basic calponin based on two diseased cases).

Additionally, as with all other blood tests, interpretation within the context of the clinical presentation and examination is needed. As entry into the present study required a high enough index of suspicion for the clinician to order some type of imaging study, the clinical suspicion for acute AD was reasonably high in control patients as well as the cases. We did not study conditions which primarily affect the gastrointestinal tract where one might suspect to also see elevations in calponin due to damaged smooth muscle. Also, since the acidic isoform of calponin is present in neurological tissue, acute neurological conditions might show elevations in this marker thus limiting the diagnostic accuracy in patients with neurological signs.

An important corollary to the possibility of having biomarkers for AD is the consideration of how such tools might interplay with diagnostic imaging. Currently, the only way to reliably diagnose acute AD is with imaging, typically either CT scanning or transesophageal echocardiography.<sup>15,16</sup> Since there is still great uncertainty how to optimize the diagnostic approach in patients suspected of having acute AD, biochemical diagnosis may improve upon this as a screening tool with emphasis on rule-out.

In conclusion, we report an initial study on the development of assays against circulating smooth muscle troponin-like protein, calponin, and its use in diagnosis of acute AD. The results of this preliminary experience using an initial assay show moderate sensitivities and specificities with negative predictive values which should be further improved upon. These findings suggest that improvement in the diagnostic performance of the assay through further technical improvements is necessary for it to be ready for production and commercial use.

**Conflict of interest:** T.S., A.D. (on behalf of the Italian/ISBEM sites), R.B., J.E.H. and K.E. received grants or consultant fees from Biosite. F.C. owned stock in Biosite.

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## Appendix

### The International Registry of Acute Aortic Dissection Substudy on Biomarkers (IRAD-Bio) Investigators

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