SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

1) **Patient flow**
2) **Use of local conventional cTn values and hs-cTnT values for adjudication of final diagnoses**
3) **Assumption of linearity of absolute changes of hs-cTnT within the first hours**
4) **Mismatches in the adjudicated diagnosis requiring involvement of a third cardiologist**
5) **Short-term changes in cardiac troponin in patients with markedly elevated levels at presentation**

SUPPLEMENTAL RESULTS

6) **Disagreement in adjudicated final diagnosis in inconsistent diagnosis of AMI**
7) **Diagnostic accuracy for the diagnosis acute myocardial infarction of the different assays**
8) **Baseline levels of hs-cTnT Roche and hs-cTnI Abbott according to final diagnosis**
9) **Subgroup analysis regarding potential pre-analytical contributors**
10) **Infarct size in patients with inconsistent AMI diagnoses**

SUPPLEMENTAL REFERENCES

TABLES

FIGURES

SUPPLEMENTAL METHODS

1. Patient flow

A detailed patient selection is shown in Figure 1S.

2. Use of local conventional cTn values and hs-cTnT values for adjudication of final diagnoses

The cTn assays used clinically in most of the participating institutions changed during the study from a conventional cTn assay to the hs-cTnT assay. In order to take advantage of the of the higher sensitivity and higher overall diagnostic accuracy offered by the hs-cTnT assay, patients were adjudicated using the hs-cTnT values in all patients. In patients in whom clinically a conventional cTn assay was used, the conventional cTn values and the hs-cTnT values were available for the adjudication. In patients in whom clinically the hs-cTnT assay was used, only the hs-cTnT values were available for the adjudication.

The following conventional cTn assays were used: For the Roche cTnT 4th generation assay, the 10% CV level is 0.035ug/l. The laboratories of the participating sites reported only two decimals; therefore 0.04ug/l was used as a cut-off for myocardial necrosis. In order to fulfil the criteria of a significant change (30% of 99th percentile or 10% CV level), a patient would e.g. need to have a level of <0.01ug/l at presentation and 0.04ug/l at 6h. A patient would also qualify if the first level is 0.02ug/l and the second 0.04ug/l. A patient would not fulfil the criteria if the first level is 0.03ug/l and the second is 0.04ug/l. If the first level is 0.04ug/l, the second level needs to be at least 0.06ug/l.

For the Abbott Axsym cTnI ADV, the 10% CV level is 0.16ug/l. A patient having 0.16ug/l at presentation would meet the criteria for significant change if the second was ≥0.21ug/l. A patient having <0.12ug/l at presentation (limit of detection) would qualify if the second is >0.16ug/l.

For the Beckmann Coulter Accu cTnI, the 10% CV level is 0.06ug/l. A patient having 0.06ug/l at presentation would qualify if the second is ≥0.08ug/l. A patient having 0.05

at presentation would qualify if the second is 0.07ug/l, but not 0.06ug/l. A patient having undetectable cTnI (cTnI<0.01ug/l) at presentation would qualify if the second is ≥0.06ug/l.

3. Assumption of linearity of absolute changes of hs-cTnT within the first hours

The assumption of linearity of absolute changes within the first hours is based on unpublished internal data as well as recent data from Ola Hammarsten et al. showing a near-linear increase in levels of hs-cTnT with increasing time from symptom onset in their NSTEMI cohort[1].

4. Mismatches in the adjudicated diagnosis requiring involvement of a third cardiologist

In our cohort of 2300 patients there were 95 (4%) mismatches in adjudicated diagnosis between the two cardiologists requiring involvement of a third cardiologist, Of these, 23 patients had the final diagnosis of AMI and 72 patients had another diagnosis. These cases were discussed in face-to-face meetings between the 3 cardiologists and a decision was reached by consensus. The same approach was followed in cases of 3 different diagnoses.

5. Short-term changes in cardiac troponin in patients with markedly elevated levels at presentation

Patients with a single markedly elevated cTn level and otherwise “clear” diagnosis of AMI including unequivocal ECG and coronary angiography findings were adjudicated to have AMI. This scenario fortunately was infrequent, but did exist. The adjudicating cardiologist at all times had the option to classify the patient to the category “chest pain of unknown cause” in case the available information was not sufficient to reliably

diagnose or exclude AMI. While the rise and/or fall criteria are important to differentiate AMI from causes of chronic cardiomyocyte damage, it is of much less importance in patients with markedly elevated cTn levels (e.g. 50-times the 99th percentile), as the positive predictive value of the markedly elevated cTn level (e.g. 50-times the 99th percentile) on its own is already above 90%[2] and approaches 100% when combined with typical ECG and coronary angiography findings. The documentation of changes of cTn is not mandatory in these patients, as the differentiation from chronic cTn elevation is not a matter of concern. Therefore, as also suggested by the ESC Biomarker study group[3], a patient with a clear AMI would still be classified AMI in case of only one markedly elevated cTn level available, as long as all other information (ECG findings, coronary angiography findings, echo findings) supported the diagnosis of AMI.

SUPPLEMENTAL RESULTS

6. Disagreement in adjudicated final diagnosis in inconsistent diagnosis of AMI

Mismatches in the adjudicated diagnosis requiring involvement of a third cardiologist in patients who were now found to have inconsistent diagnosis of AMI related to biological non-equivalent CDVs:

- hs-cTnT Roche and hs-cTnI Abbott: 11/86 (12.8%)
- hs-cTnT Roche and s-cTnI Siemens: 10/78 (12.8%)
- s-cTnI Siemens and hs-cTnI Abbott: 0/32 (0%)
- hs-cTnI Abbott and hs-cTnI Siemens: 7/46 (15.2%)
- hs-cTnI Abbott and hs-cTnI Beckmann: 1/32 (3.1%)
- hs-cTnT Roche and hs-cTnI Siemens: 1/31 (3.2%)
- hs-cTnT Roche and hs-cTnI Beckmann: 2/24 (8.3%)

7. Diagnostic accuracy for the diagnosis acute myocardial infarction of the different assays

Informations about diagnostic accuracy of all the used c-Tn assays provided in table 1S.

8. Baseline levels of hs-cTnT Roche and hs-cTnI Abbott according to final diagnosis

In hs-cTnI Abbott, 15.4% of baseline values were below the LOD of 1.9ng/L, 57.9% were in the range between LOD and the 99th percentile of 26.2ng/L (73.3% below 99th P) (Figure 2S). Corresponding percentages in hs-cTnT Roche were 30.1% below LOD of 5ng/L and 35.3% between LOD and 99th percentile of 14ng/L (65.5% below 99th P). In conclusion, there were more of hs-cTnI Abbott values detectable, but in general the assay was less predictive for AMI than hs-cTnT Roche.

9. Subgroup analysis regarding potential pre-analytical contributors

In 1355 patients parallel measurements of hs-cTnT and s-cTnI-ultra (Siemens) were performed from the same tube and on the same day. In this subgroup findings were similar to that of the overall cohort. AMI was the adjudicated diagnosis in 294 patients (22%). Among patients with an adjudicated diagnosis of AMI levels of hs-cTnT and s-cTnI levels correlated closely ($r=0.802$, $p<0.001$). Among these, 53 patients (18%) had inconsistent diagnoses using the approved uniform CDV. Nearly all of these inconsistencies were related to under-diagnosis of AMI with s-cTnI-ultra. Using the s-cTnI-ultra biological-equivalent CDV for hs-cTnT (9.4ng/l instead of 40ng/l) reduced inconsistencies regarding the diagnosis of AMI from 19% to 9% ($p<0.001$). In 101

patients measurements of hs-cTnI and hs-cTnT were performed from fresh samples. Again, findings were similar to that in the overall cohort (Figure 3S).

10. Infarct size in patients with inconsistent AMI diagnoses

Median c-Tn values in patients with inconsistent diagnoses of AMI were lower than in patients with consistent AMI diagnoses, indicating that infarct size was smaller in patients with inconsistent diagnoses (Table 2S). This result was consistent in all comparisons between the different assays.

SUPPLEMENTAL REFERENCES

1. Hammarsten O, Fu ML, Sigurjonsdottir R, Petzold M, Said L, Landin-Wilhelmsen K, Widgren B, Larsson M, Johanson P. Troponin T percentiles from a random population sample, emergency room patients and patients with myocardial infarction. *Clin Chem* [Internet]. 2012;58:628–637. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22258764
2. Reichlin T, Schindler C, Drexler B, Twerenbold R, Reiter M, Zellweger C, Moehring B, Ziller R, Hoeller R, Rubini Gimenez M, Haaf P, Potocki M, Wildi K, Balmelli C, Freese M, Stelzig C, Freidank H, Osswald S, Mueller C. One-hour rule-out and rule-in of acute myocardial infarction using high-sensitivity cardiac troponin T. *Arch Intern Med* [Internet]. 2012 [cited 2013 Mar 14];172:1211–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22892889
3. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, Huber K, Plebani M, Biasucci LM, Tubaro M, Collinson P, Venge P, Hasin Y, Galvani M, Koenig W, Hamm C, Alpert JS, Katus H, Jaffe AS, the Study Group on Biomarkers in Cardiology of the ESCWG on ACC. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Hear J* [Internet]. 2012;Available from: http://www.ncbi.nlm.nih.gov/pubmed/22723599

Tables

Table 1S: Diagnostic accuracy for the diagnosis acute myocardial infarction of the different assays

	Sensitivity (95%Cl)	Specificity (95%Cl)	PPV (95%Cl)	NPV (95%Cl)	Accuracy
hs-cTnT Roche	89.9% (86.8-92.4)	79.8% (77.9-81.6)	53.5% (50.0-57.0)	96.8% (95.8-97.6)	81.9%
hs-cTnI Abbott	72.9% (68.7-76.9)	92.8% (91.5-93.9)	72.3% (68.1-76.3)	93.0% (91.7-94.1)	88.7%
hs-cTnI Siemens	92.4% (88.5-95.3)	74.1% (71.4-76.6)	45.2% (40.9-49.5)	97.7% (96.4-98.6)	77.5%
hs-cTnI Beckman	91.6% (86.9-95.1)	74.9% (71.8-77.7)	46.0% (41.1-51.0)	97.5% (95.9-98.5)	78.0%
s-cTnI Siemens	75.7% (71.5-79.5)	91.7% (90.4-93.0)	70.6% (66.3-74.6)	93.5% (92.2-94.6)	89.4%

Table 2S: median c-Tn values in ng/L during serial sampling in patients with consistent compared to inconsistent diagnosis of AMI

	hs-cTnT Roche and hs-cTnI Abbott		s-cTnI Siemens and hs-cTnT Roche		s-cTnI Siemens and hs-cTnI Abbott		hs-cTnI Siemens and hs-cTnI Abbott	
median, in ng/L	hs-cTnT Roche	hs-cTnI Abbott	s-cTnI Siemens	hs-cTnT Roche	s-cTnI Siemens	hs-cTnI Abbott	hs-cTnI Siemens	hs-cTnI Abbott
consistent diagnosis	108.3	413.7	104.4	595.5	425.5	253.6	785.1	601.4
inconsistent diagnosis	28.9	19	28.4	29.3	61.5	29.6	36.6	28.8
p for comparison	<0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001

	hs-cTnI Beckman and hs-cTnI Abbott		hs-cTnT Roche and hs-cTnI Siemens		hs-cTnT Roche and hs-cTnI Beckman	
median, in ng/L	hs-cTnI Beckman	hs-cTnI Abbott	hs-cTnT Roche	hs-cTnI Siemens	hs-cTnT Roche	hs-cTnI Beckman
consistent diagnosis	470	612.4	126.8	578.4	131.1	367.4
inconsistent diagnosis	27.7	38.2	29.8	30.5	29.4	19.9
p for comparison	<0.001	<0.001	<0.001	<0.001	0.001	<0.001

Figures

Figure 1S: Selection of the study population

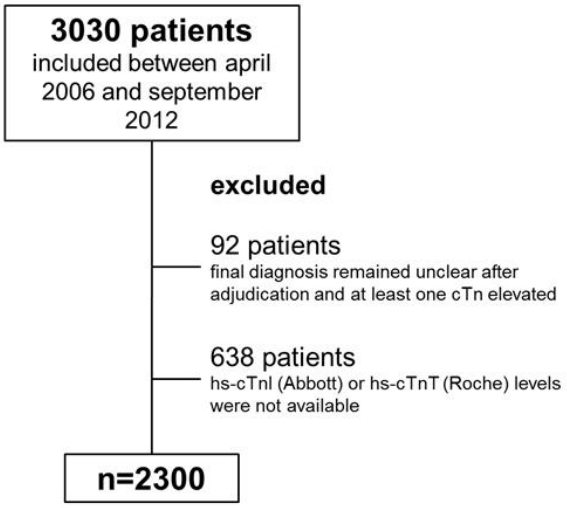

Figure 2S: baseline levels of hs-cTnT Roche and hs-cTnI Abbott according to final diagnosis

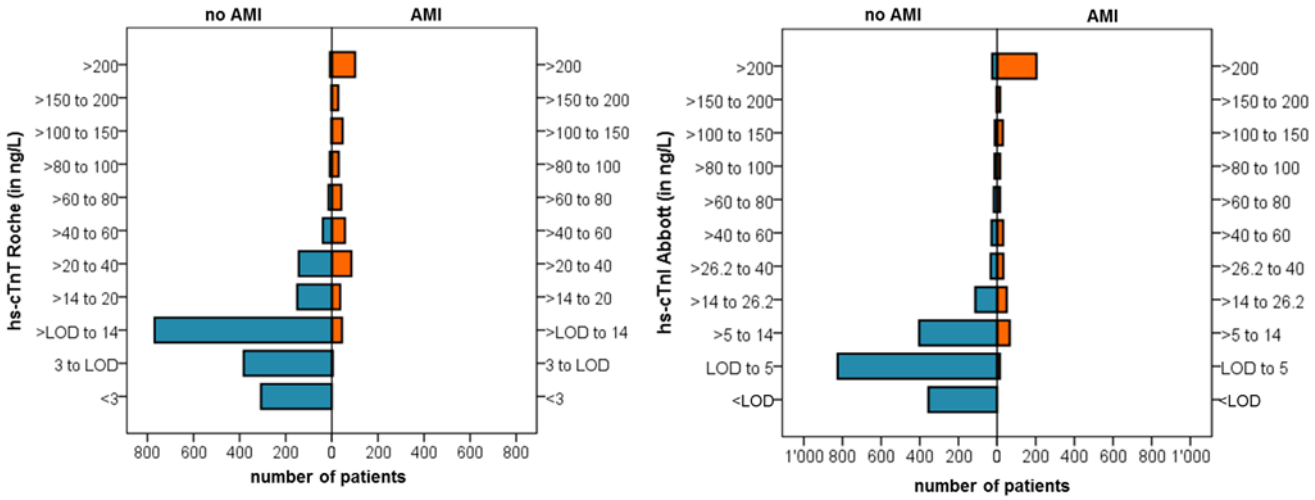

Figure 3S: Measurements in fresh samples confirm that the approved clinical decision values (red lines) for the two assays are not biological-equivalent