Arguments supporting the benefits and advantages of human

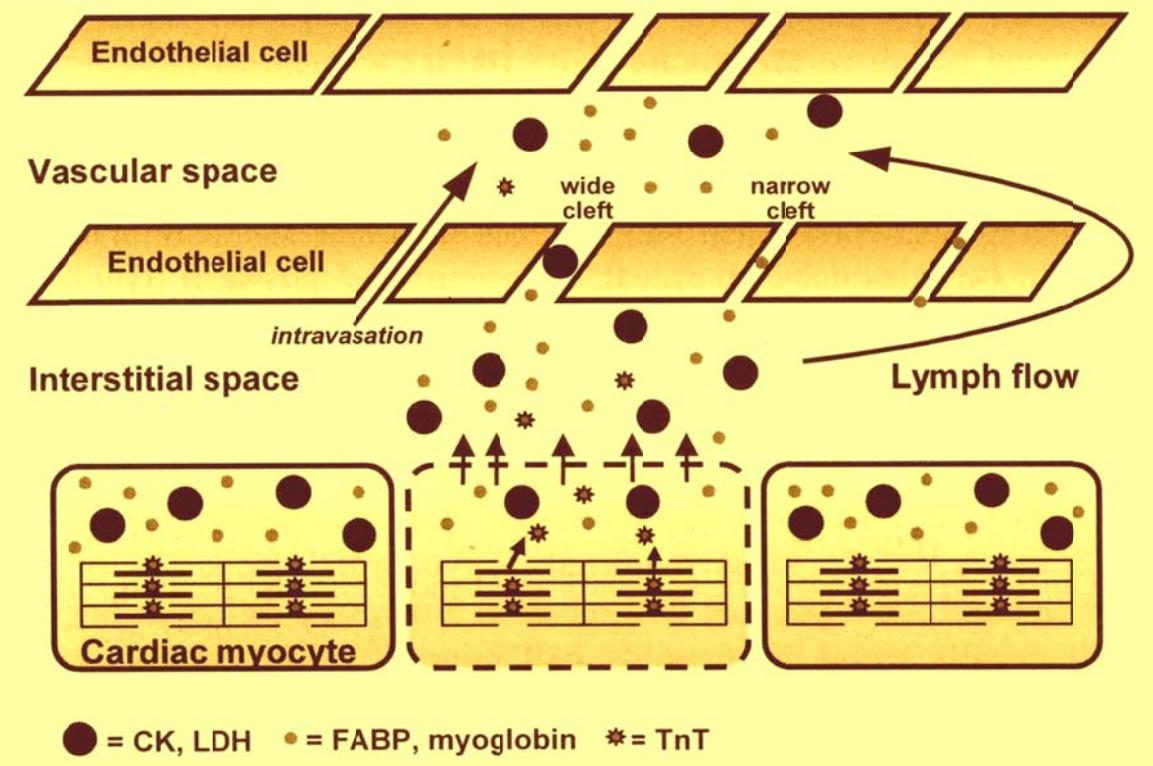
heart-type fatty acid-binding protein (h-FABP)

as early marker for acute myocardial infarction (AMI)

When minutes count …

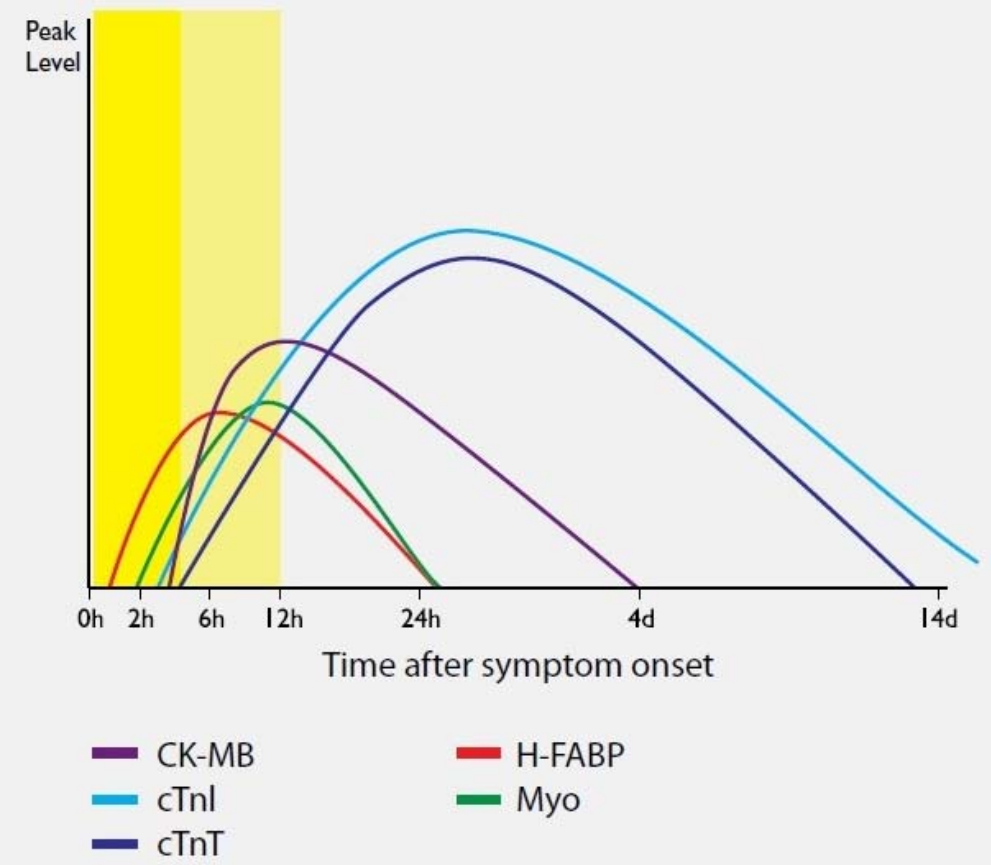
h-FABP is a small protein (15 kDa) abundantly present in myocardial cells. It is involved in the intracellular uptake and transport of long-chain fatty acids. Due to the small size **and** the cytoplasmic localisation it is rapidly released into the circulation after myocardial cell damage.

The possible transport routes of proteins released from damaged cardiac myocytes to the plasma compartment are shown below. Proteins can either cross the endothelial cell barrier directly (predominant route for **small proteins** such as **h-FABP** and Myoglobin) or they can be transported through lymph drainage (predominant route for larger proteins such as Creatine-Kinase (CK) and Lactate Dehydrogenase (LDH)). **Structurally bound proteins** (such as **Troponins**) **must be dissociated** first from the myofibrillar structures before they can be released into the interstitial space[1].



[1] Van der Voort D: Development of an immunosensor for on-line continuous measurement of cardiac injury. Thesis (2003), Maastricht University, 16

Based on the release mechanism the following time course can be observed for the different cardiac markers after AMI[2]:



The diagnostic potential of h-FABP was first reported by Prof. Glatz (Maastricht University, The Netherlands) in 1988[3]. In the meantime many studies have confirmed that **h-FABP** is a **promising early marker of myocardial damage** and **improves diagnosis of AMI** and **risk stratification**.

The h-FABP advantages in a nutshell:

- Early release due to cytoplasmic location
- Therefore, detectable as early as 30 minutes after ischemic episode[4]
- Rapid increase from base levels to clinical cut-off value, even faster than Myoglobin[5]
- Furthermore, low normal plasma value
- Extremely stable protein

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[2] Data source: http://www.randox.com/brochures/PDF%20Brochure/LT237.pdf
[3] Glatz JFC, van Bilsen M, Paulussen RJA, et al.: Biochem Biophys Acta (1988), 961, 148-52
[4] Kleine AH, Glatz JF, van Nieuwenhoven FA, van der Vasse GJ: Release of heart type fatty acid binding protein into plasma after acute myocardial infarction in man. Mol Cell Biochem (1992), 116, 155-162
[5] Pelsers MM, Hermens WT, Glatz JF: Fatty acid-binding proteins as plasma markers for tissue injury. Clin Chem Acta (2005), 352 (1-2), 15-35

Particularly in the first hours after onset of AMI symptoms h-FABP is superior compared to contemporary Troponin (I) assays[6] (NPV = negative predictive value; AUC area under the ROC curve):

Sensitivity	0-3 hrs	3-6 hrs	6-12 hrs	12-24 hrs
cTnI	50%	68%	81%	96%
H-FABP	64%	85%	90%	90%
Specificity	**0-3 hrs**	**3-6 hrs**	**6-12 hrs**	**12-24 hrs**
cTnI	93%	94%	94%	94%
H-FABP	84%	89%	94%	91%
NPV	**0-3 hrs**	**3-6 hrs**	**6-12 hrs**	**12-24 hrs**
cTnI	92%	95%	97%	99%
H-FABP	93%	97%	98%	99%
AUC	**0-3 hrs**	**3-6 hrs**	**6-12 hrs**	**12-24 hrs**
cTnI	0.76	0.85	0.90	0.98
H-FABP	0.84	0.89	0.94	0.97

In order to reduce the "Troponin-blind phase" at the beginning of a heart attack **high sensitive assays** were developed in the recent past. The clinical sensitivity of the mentioned tests is much higher than for the contemporary Troponin assays, especially in the early phase of an AMI. However, the **increased sensitivity** (95 %) comes along with a **reduced clinical specificity** (80 %) and a lower positive predictive value (PPV 50 % (NPV 95 %))[7]. Before reducing the test-specific cut-off (e.g. from 100 to 14 pg/ml for Troponin T) it was an established doctrine that Troponins (T and I) are absolutely specific markers for cardiac muscle damage and AMI. With the high sensitive assays the lower measuring range was expanded into regions which were not detectable a short while ago. This fact is caused by the problem that the **very low Troponin concentrations** measureable now (picogram range → down to 0.000 000 000 014 grams/ml), can **have other origin than AMI**. Slightly increased Troponin levels may also be due to: abnormally fast heart beat, high blood pressure in lung arteries (pulmonary hypertension), blockage of a lung artery (by a blood clot, fat, or tumour cells (pulmonary embolus)), congestive heart failure, coronary artery spasm, inflammation of the heart muscle usually due to a virus (myocarditis), strenuous exercise (for example, due to marathons or triathlons), trauma that injures the heart such as a car accident, weakening of the heart muscle (cardiomyopathy). Increased Troponin levels may also result from certain medical procedures such as: cardiac angioplasty/stenting, heart defibrillation or electrical cardioversion (purposeful shocking of the heart by medical personnel), open heart surgery and radiofrequency ablation of the heart. This

[6] McMahon CG, Lamont JV, Curtin E, McConnell RI, Crockard M, Kurth MJ, Crean P, Fitzgerald SP: Diagnostic accuracy of heart-type fatty acid-binding protein for the early diagnosis of acute myocardial infarction. Am J Emerg Med (2012), Feb 30(2), 267-74

[7] Reichlin *et al.*, N Eng J Med (2009), 361, 858-67

loss of clinical specificity was clearly **verified** at the congress of the American Association for Clinical Chemistry (AACC) in 2010. Ferreira C.E. *et al.* from the Hospital Albert Einstein, São Paulo, Brazil have presented results from a clinical study with the objective: to evaluate the routine Troponin I in a general hospital and correlate it with clinical situations (Poster C-35). 4.559 Troponin I tests were done and 1.540 tests (33.78 %) had values above the high sensitive cut-off point (34 pg/ml for Troponin I). The main differential diagnosis of patients with test results above the cut-off was composed as follows:

- ACS : 25%
- Cardiac surgery : 7.8%
- Cardiac catherization : 2.4%
- Angioplasty : 5.2%
- Heart failure- NO AMI : 7.7%
- Chest pain : 7%
- Arrhytmia : 5.7%
- Respiratory failure : 5.7%
- Non cardiac surgery : 5%
- Sepsis : 4.3%
- Infectious processes : 3.4%
- Acute pulmonary edema : 2.7%
- Hypertension, pulmonary thromboembolism, kidney failure death : 0-2% each
- Cancer, transplantation, diabetes, and other disease situations : 13%

These data demonstrate the loss of clinical specificity impressively. Similar results were published from Koerbin G. *et al.*[8]. **A cardio-healthy** reference **population** was investigated using the new **high sensitive Troponin T** assays. **Approximately 42%** of the samples showed Troponin T **concentrations above** the manufacturer's quoted **limit of detection**. The authors conclude that with many apparently healthy people having detectable Troponin, clinical judgement will become more important in interpreting Troponin results.

For the high and ultra-high sensitive Troponin assays it can be summarized:

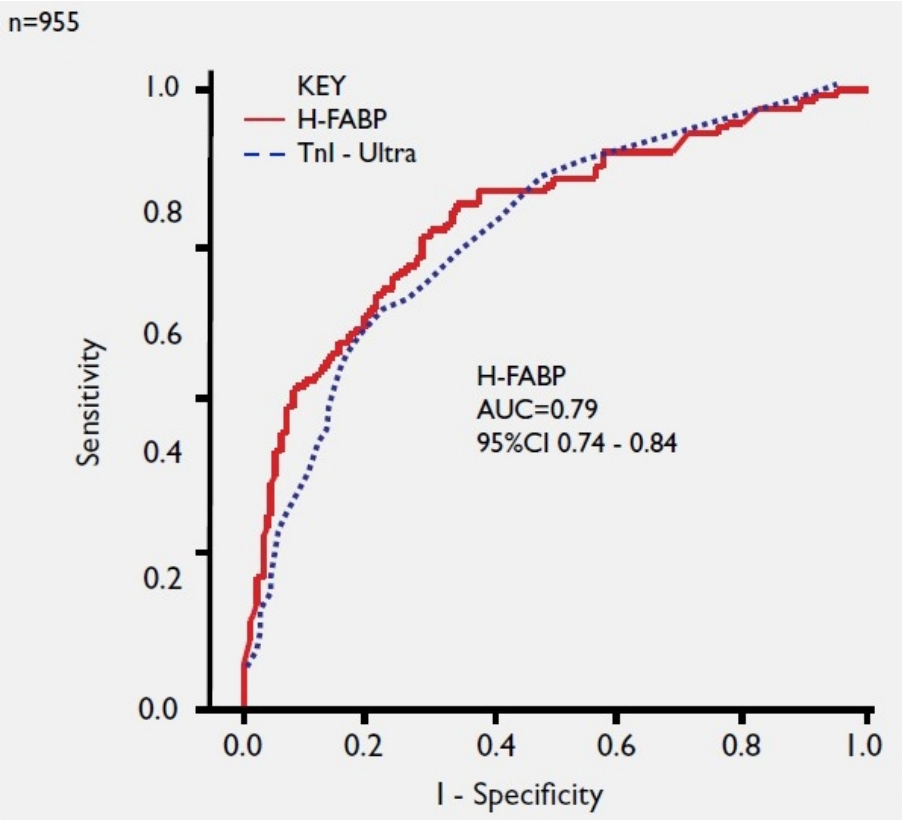
increased sensitivity leads to decreased clinical specificity

and this is the other side of the coin with respect to the challenge of cut-off optimization.

However, there is no need to accept a loss of specificity in the first hours after onset of AMI symptoms. The solution to this problem has a name: h-FABP!

[8] Koerbin G *et al.*, Annals of Clinical Chemistry (2010), 47, 524-28

The **benefits of h-FABP** are **evident**, even when used **in addition to a high sensitive Troponin** assay (Siemens Advia Ultra-TnI) that meets the ACC/ESC guidelines. Viswanathan *et al.*[9] have shown that the receiver-operator curves (ROC) for h-FABP and ultra-TnI in the prediction of death or AMI have a significantly higher area under the curve for h-FABP (0,79) than for ultra-TnI (0,77).



Attention should also be paid to the fact that a combination of h-FABP and Troponin (I) can be used effectively as rule-out test to exclude AMI within 6 hours of pain onset.

In a study with a total of 1128 patients (providing 2924 venous blood samples) McMahon *et al.*[10] have evaluated the diagnostic efficacy of multiple tests – heart-type fatty acid–binding protein (h-FABP), cardiac troponin I (cTnI), creatine kinase-MB, and myoglobin – for the early detection of acute myocardial infarction among patients who present to the emergency department with chest pain. The results can be summarized as follows:

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[9] Viswanathan K, Kilcullen N, Morrell C, Thistlethwaite SJ, Sivananthan MU, Hassan TB, Barth JH, Hall AS: heart-type fatty-acid binding-protein (H-FABP) predicts long-term mortality and re-infarction in consecutive patients with suspected acute coronary syndrome who are troponin negative. J Am Coll Cardiol (2010), 55 (23), 2590-8

[10] McMahon CG, Lamont JV, Curtin E, McConnell RI, Crockard M, Kurth MJ, Crean P, Fitzgerald SP: Diagnostic accuracy of heart-type fatty acid-binding protein for the early diagnosis of acute myocardial infarction. Am J Emerg Med (2012), Feb 30(2), 267-74

- **h-FABP** had the **greatest sensitivity** at 0 to 3 hours (64.3%) and 3 to 6 hours (85.3%) after chest pain onset
- the **combination** of **cTnI** measurement with **h-FABP increased sensitivity** to 71.4% at 3 to 6 hours and 88.2% at 3 to 6 hours
- receiver operating characteristic curves demonstrated that **h-FABP** had the **greatest diagnostic ability** with area under the curve at 0 to 3 hours of 0.841 and 3 to 6 hours of 0.894
- the **specificity** was also **high for** the **combination** of h-FABP with cTnI at these time points
- **h-FABP** had the **highest negative predictive values** of all the individual markers: 0 to 3 hours (93%) and 3 to 6 hours (97%)
- the **combined measurement** of cTnI with h-FABP **increased** the **negative predictive values** to 94% at 0 to 3 hours, 98% at 3 to 6 hours, and 99% at 6 to 12 hours.

Therefore, it can be concluded that **testing both** h-FABP and cTnI provides a reliable diagnostic tool for the early diagnosis of myocardial infarction/acute coronary syndrome and also a **valuable rule-out test** for patients presenting at 3 to 6 hours after chest pain onset.

	NPV in %			
Time post pain	0-3h	3-6h	6-12h	12-24h
Individual markers				
H-FABP	93	97	98	99
cTnI	92	95	97	99
2 marker combinations				
H-FABP + cTnI	94	98	99	100

Time saves heart muscle …

Against this background it should be mentioned that the high and ultra-high sensitive Troponin assays are dependent on (modular) analysers, which are not suitable for point of care and bedside diagnostic.

In contrast the lateral flow device **QuickSens®h-FABP** fulfilled the requirements of the primary care market with respect to:

- available equipment,
- **time-to-result** and
- simplicity of workflow.