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Point of Care tests for the diagnosis of NASH in clinical trials, design consideration and potential use

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Issues in evaluating the performance of POC for the diagnosis of NASH

- Choice of the right reference method (« Gold Standard »)
- Choice of the best surrogate marker
- Design of the POC test for the surrogate marker
- Choice of the right methodological approach for evaluating the performance of the POC test in clinical trials

Reference methods for the diagnosis of NASH (1)

- « Gold standard » for years: liver biopsy and use of Kleiner classification[1]
- New imaging methods avoiding liver biopsy:
  - Combination of methods identifying NAFL and liver fibrosis
    - NAFL:
      - Ultrasonography for steatosis[2]
      - Proton Magnetic Resonance Spectroscopy[3]
      - Liver MRI[4]
      - CAP sofware implemented in TE
    - Liver fibrosis
      - Transient elastometry for fibrosis[5]

[1]Kleiner, Hepatol 2005. [2]Hamagushi, Am J gastroenterol 2007. [3]Szczepaniak LS. Am J Physiol Endocrinol Metab 2004. [4]Donato, Eur J Radio, 2017. [5]Koehler, Hepatol 2016.

Reference methods for the diagnosis of NASH (2)

- New biochemical methods avoiding liver biopsies:
  - here again, must combine diagnosis of steatosis and fibrosis

Table 1 Accuracy of the most-well studied serologic markers for detecting steatosis and for differentiating simple steatosis from nonalcoholic steatohepatitis

Marker	AUROC	*n*	Ref.
Serologic markers for detecting steatosis			
CK18	0.90	157	[20]
	0.77	318	[21]
FLI	0.84	496	[27]
LAP	0.79	588	[28]
Hepatic steatosis index	0.81	5362	[29]
SteatoTest	0.79	69	[31]
Serologic markers for differentiating simple steatosis from nonalcoholic steatohepatitis			
APRI	0.60	190	[34]
CK18	0.82	838	[39]
NAFLD fibrosis score	0.82	733	[43]
Comparative studies of serologic markers for differentiating simple steatosis from nonalcoholic steatohepatitis			
FIB-4	0.86	145	[46]
NAFLD fibrosis score	0.81		
BARD score	0.77		
APRI	0.67		
FIB-4	0.96	165	[47]
NAFLD fibrosis score	0.94		
BARD score	0.84		
FIB-4	0.80	541	[48]
NAFLD fibrosis score	0.77		
BARD score	0.70		
APRI	0.73		
FIB-4	0.87	576	[49]
NAFLD fibrosis score	0.86		
APRI	0.79		
BARD score	0.76		

Papagianni, World J Hepatol 2015

Reference methods for the diagnosis of NASH (3)

- GPR (GGT/Platelets ratio), new fibrosis score evaluated in chronic hepatitis B[1] and HIV-infection with HBV[2]
- Never formally evaluated in NAFL patients assessed for fibrosis
- Personal data, from a database of 1185 Patients (J. Boursier)

Test	AUROC	
	Fibrose septale	Cirrhose
FIB4	0.823 ± 0.014	0.890 ± 0.019
APRI	0.786 ± 0.016	0.847 ± 0.022
GPR	0.742 ± 0.016	0.858 ± 0.024

[1]Lemoine, Gut 2017. [2]Boyd, Gut 2017

Major limits of surrogate markers

- Performance of biochemical scores is not 100% (AUROC of 0,70 to 0,90, influenced by intercurrent health hazards)
  - Should we consider this level of performance enough for clinical trials?
- Most surrogate markers differentiate between cirrhosis and no cirrhosis, but is it enough ?
- None has been evaluated in patients under treatment for NASH
  - Ex: not reliable enough in longitudinally evaluating fibrosis in patients treated for chronic hepatitis
  - In clinical trials, what would be the right endpoint: decrease in 1 point of a marker? ➔ clinical significance +++
- None can be considered a Point of Care test

Definition of a Point of Care Test

- "Portable devices meant to perform diagnostic assays at or near the site of patient care"
- Aim to provide same-day diagnosis and facilitate immediate decision-making
- ASSURED criteria defined by WHO
  - **A**ffordable
  - **S**ensitive
  - **S**pecific
  - **U**ser-friendly
  - **R**apid and robust,
  - **E**quipment-free
  - **D**eliverable to end users

POC tests for NAFL and fibrosis in NASH

- Portable TE: considered as a POC device, but can only diagnose fibrosis (the CAP sofware can be added, but accuracy?) ➔ should be combined with a screening test for steatosis

- Simple biochemical scores (APRI, FIB-4, LFI, etc.) ➔ could be considered as POC scores IF use of POC devices to measure the biochemical parameters needed in the score

Example of POC tests for biochemical markers (1)

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journal homepage: www.elsevier.com/locate/bios

Advances in paper-based point-of-care diagnostics

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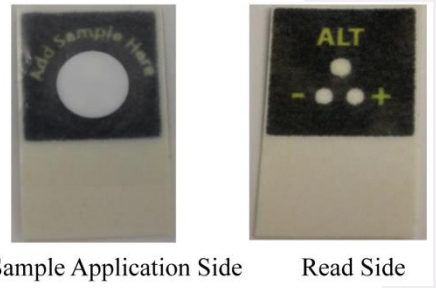
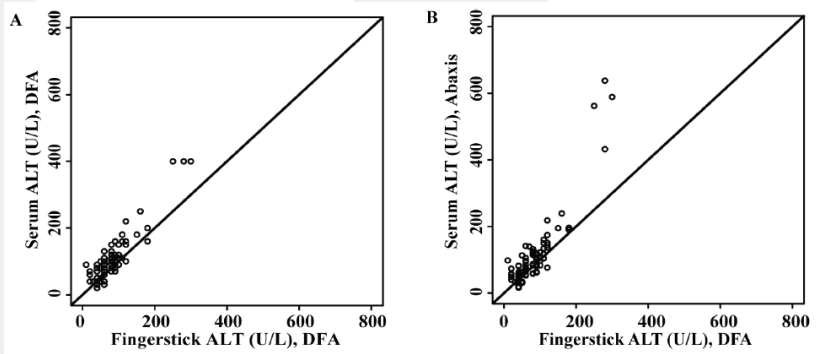
- Paper-based diagnostic technology: affordable, user-friendly, rapid, robust, and scalable for manufacturing
- Based on three main technologies: dipstick assays, lateral flow assays and microfluidic paper-based diagnostics
- One example of a validated test for a rapid visual measurement of ALT[1]:
  - Peroxydase-based colorimetric assay providing semi-quantitative ALT: N, 3-5ULN, >UL5N

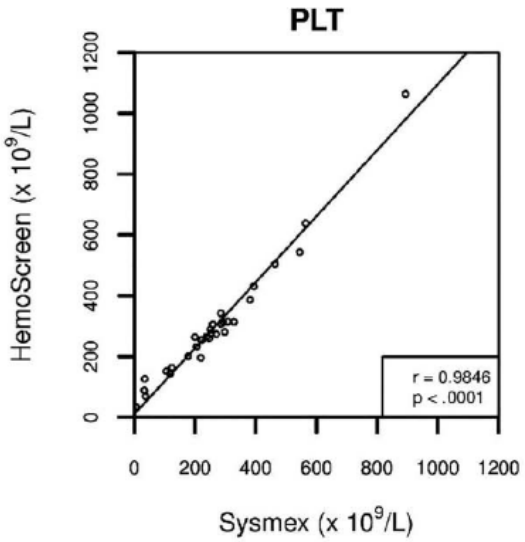




[1]Jain S, PlosOne 2015

Example of POC tests for biochemical markers (2)

- Platelet count for the calculation of APRI or FIB-4
- New technology combining flow cytometry and digital imaging in a single platform ➔ employs a novel method called viscoelastic focusing which aligns the cells in a single plane[1]



[1]Ben-Josef, J Cin Pathol 2016

Accuracy of surrogate markers to be used in clinical trials

- Are the performance of surrogate markers for NAFL and fibrosis in NASH universal?
- Example of HIV-infected patients, the ECHAM study (n=402):
  - Cross-sectional evaluation of the prevalence of NAFLD/NASH in HIV-infected patients with a dysmetabolic profile[1]
  - Use of liver MRI as a gold standard
  - Surrogate markers used : ET+ CAP, FibroMax
  - If discrepancy between two markers of fibrosis, liver biopsy performed

[1]Lemoine, EASL 2017

Results of the ECHAM study (49 patients with LB)

	n=49
Length of liver biopsy	16.4 +/- 5 mm
NAFLD	37 (76%)
NAFL (steatosis only) NASH	14 (29%) 23 (47%)
Fibrosis F0/F1 Fibrosis F2-4 F2 F3 Cirrhosis (F4)	31 (67%) 15 (33%) 7 (15.2%) 6 (13%) 2 (4.3%)

	Methods	False positive	False negative	True positive	True negative	Pos. Likelihood ratio	Neg. Likelihood ratio	Sensitivity	Specificity
Fibrosis	Fibroscan (n=43)	19	3	12	9	1.18	0.62	0.80	0.32
	Fibrotest (n=49)	19	4	11	15	1.31	0.60	0.73	0.44
Steatosis	MRI (n=49)	5	2	21	21	4.75	0.11	0.91	0.81
	Steatotest (n=49)	10	6	17	16	1.92	0.42	0.74	0.62
NASH	NashTest (n=49)	6	13	2	28	0.76	1.05	0.13	0.82

How to use POC tests in clinical trials on NASH treatment ?

- At this stage, the use of a reference method is still mandatory
- A clinical trial is the perfect setting for the evaluation of a POC strategy (validation study) in parallel to the evaluation of treatment efficacy and tolerability

WHICH POC-BASED STRATEGY?

Road map – Next steps

- Designing a real POC score based on POC devices and POC tests
- Choosing the right Gold Standard method (LB may remain the reference method until a POC strategy is formally evaluated)
- Designing a validation study of a POC-based strategy for evaluating steatosis and fibrosis in NASH patients along with the evaluation of treatment efficacy and tolerability
  - Limits of validity of surrogate markers for longitudinal assessment
  - Choice of POC tests?

- Call for consensus