Evidence Based Diagnostic In Microbiology

UCL *Séminaire de Pathologie Infectieuse*

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Cliniques Universitaires Saint-Luc, Bruxelles

Dr M. Ieven

Laboratorium voor Microbiologie
Universitair Ziekenhuis Antwerpen
en Universiteit Antwerpen

Evidence Based Diagnostic Microbiology

= Part of Evidence based Medicine

“Evidence-based medicine is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients”

Sackett et al, 1996

Evidence based Microbiological Diagnosis

Current practice in decision making:

- tradition (standard operation) e.g. cold agglutinins, Widal serology
- anecdotal ("an identical case e.g. HCV in sarcoidosis...")
- one publicaton ("the authors recommend…")
- experts advice ("in my experience…")
- financial (expensive procedure is not an improvement)
- through search for, critical evaluation of and correct use of proven procedures (= **evidence based**)

Decisions and Implementation of Evidence Based Diagnosis

"Conscientious and judicious use"

⇒ evidence of no value : eliminate

⇒ necessity for rational cost control

- cost control not aimed at savings per se but at efficient use of available means, replacing obsolete or tests with no added value, by judiciously applied improved technology

Critical Appraisel about Evidence Based Diagnostics

- Is the evidence about the accuracy of the diagnostic test valuable?

  ⇒ Validation of the diagnostic test

- What is the impact/importance of the test : can the test accurately distinguish patients with this disease ?

  ⇒ predictive value of the test e.g. HIV test-versus Borrelia Ab, Legionella IgM

- Applicability: can we use this valid and clinically important test for this patient population ?

Evidence Based Diagnostic Microbiology

- Validation of diagnostic tests
- Utility of diagnostic tests in clinical practice
  - evidence based restriction rules for routine tests
    - stool cultures
    - sputum gram and culture
    - HSV molecular tests in CSF
    - MTB molecular tests
  - screening strategies : *C. trachomatis*
  - detection of novel pathogens in chronic diseases

Guides for Deciding the Clinical Usefulness of a Diagnostic Tests (I)

- Has there been a “blind” comparison with the best available reference test or “gold standard” ?
- Has the test been evaluated in a patient sample including the spectrum of mild, severe, (treated and untreated) disease and individuals with different but commonly confused disorders ?
- Was the setting and selection of patients adequately described ?

Sackett et al, 1996

Guides for Deciding the Clinical Usefulness of a Diagnostic Tests (II)

- Has the reproducibility of the test (precision) and its interpretation (observer variation) been determined ?
- Has the utility i.e. contribution to the diagnosis and/or treatment, clinical outcome been determined ?
- If the test is advocated as part of a cluster or sequence of tests, has its individual contribution to the overall been determined ?

Sackett et al, 1996

Nucleic Acid Amplification Techniques

- Commercialized tests
  - extensive validation and standardization
- Only a few FDA cleared kits
  - HIV, *M. tuberculosis*, *C. trachomatis*, *N. gonorrhoeae*, HPV, HCV
- Majority require use of in-house developed methods
  - restricted availability
  - degree of validation and standardization is often not transparent or even lacking

Blind Comparison with Reference test : "Discrepancy in Discrepant Analysis"

- difficult to apply if sensitivity new test > sensitivity ref test

		reference test +	-
new test	+	a	b
	-	c	d

- apparent false positive specimens (b)= retested or confronted with clinical information to move them to (a)
- much larger group (d) not retested, although some could be positive after retesting

Hadgu A. Lancet 1996; 348: 592-593

Strategy for Validation of New Molecular Tests

- retesting not restricted to discrepant specimens
- **expanded gold standard**[1] : confirmation of a positive PCR result by a second PCR amplifying another part of the genome, or by another amplification technique
- **latent class analysis**[2] : by a battery of independent tests (minimum 3), sensitivity and specificity of each test can be provided without an absolute reference test

(1) Toye R et al. J. Clin. Microb. 1996; 34: 1396
(2) Qu Y et al. Biometrics 1996; 52: 797-810

LCA Evaluating Autolysin PCR and Pneumolysin PCR of Sputum for Diagnosis of Pneumococcal Pneumonia.

Model	Sensitivity (95% CI)	Specificity (95% CI)
A		
Blood culture	29 (0-64)	100 (100-100)
Sputum gram stain	52 (17-86)	84 (69-99)
ICG urine antigen test	77 (55-99)	71 (40-100)
Autolysin PCR	82 (65-100)	38 (20-55)
B		
Blood culture	36 (0-73)	100 (100-100)
Sputum gram stain	56 (27-85)	83 (69-98)
ICG urine antigen test	78 (58-99)	67 (46-87)
Pneumolysin PCR	89 (70-100)	27 (15-39)

NOTE. Model A, goodness-of-fit $\chi 2$, 2.87 (P =.83); model B, goodness-of-fit $\chi 2$, 3.82 (P =.70).
CI, confidence interval; ICG, immunochromatographic assay (NOW *Streptococcus pneumoniae*; Binax)

Butler JC et al. J. Infect. Dis.2003; 187: 1422

Detection of Rhinovirus in Nasopharyngeal Aspirates: Comparison of Culture-NASBA and PCR Results based on EGS and LCA (N = 520)

		EGS (%)	LCA (%)	(95%) (CI)
Culture	**Se**	**34.1**	**28.1**	(15 - 41)
	Sp	98.7	99.2	(98 - 100)
Nasba	**Se**	**87.2**	**82.1**	(60 - 100)
	Sp	98.3	99.8	(98 - 100)
PCR	**Se**	**85.1**	**77.9**	(63 - 93)
	Sp	93.4	94.5	(91 - 97)

EGS: Nasba-PCR: No significant difference

LCA: Nasba-PCR: significant difference

Utility of Diagnostic Tests

- Number of laboratory tests increases steadily: with 4.5 - 9.5% in appropriate ordening

  Van Walraeven, JAMA, 1998; 280: 550

- Within appropriate requests, there is an overuse of the excisting diagnostic tests.

⇒ May result in increase of false positive or false negative results, further investigations and patient disconfort.

⇒ Necessity for restriction rules !!

Evidence Based Diagnostic Microbiology

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Selective Criteria for the Microbiological Examination of Faecal Specimens

- "3 day-rule": eliminate routine stool cultures of patients hospitalised > 3 days
  - ⇒ results in 30[(1)] - 50%[(2)] workload reduction on these specimens
  - ⇒ results in significant reduction of hospital and patient costs without altering patient care

[(1)] Siegel et al., JAMA 1990; 263: 979

[(2)] Fan et al, J. Clin. Microbiol. 1993; 31: 2233

- "5 day-rule": reason: 3 day-rule would have missed
  12 cases/854 specimens
  5 day-rule would miss only 3 cases /854

Hanscheid et al., Clin. Microbiol. Infect. 2002; 8: 118-21

Categories Indicating the Strength of Recommendations and the Quality of Evidence on which they are based.

Strength of evidence	
A	Good evidence to support a recommendation for use
B	Moderate evidence to support a recommendation for use
C	Poor evidence to support a recommendation for or against use
D	Moderate evidence to support a recommendation against use
E	Good evidence to support a recommendation against use
Quality of evidence	
I	Evidence from at least one properly randomized, controlled trial
II	Evidence from at least 1 well-designed clinical trial without randomization, f rom cohort or case-controlled analytic studies, from multiple time-series studies, or from dramatic results in uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Guerrant R et al, Clin. Infect. Dis. 2001; 32: 331-348

Evidence Based Selective Fecal Studies: Evidence Ranking BII

Community Acquired or traveler's diarrhea

Culture or test for:

Salmonella

Shigella

Campylobacter

***E. coli* 0157:H7 (if blood in stool also test for Shiga toxin)**

***C. difficile* toxins A ± B (if recent antibiotics)**

Nosocomial diarrhea

(onset after > 3 d in hospital)

Test for

***C. difficile* toxins A ± B**

(in suspect nosocomial outbreaks, in patients with bloody stools, and in infants, also add tests (in panel A)

Persistent diarrhea >7d

(esp. if immunocompromised)

Consider parasites

Giardia

Cryptosporidium

Cyclospora

Isospora belli

Guerrant R et al, Clin. Infect. Dis. 2001; 32: 331-348

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Sputum Culture in Untreated Cases of Definite Pneumococcal Pneumonia

Study	n	Reference Standard	Positive Culture (%)
Fiala	25	Blood culture	14/25 (56)
Barret-Connor	33	Blood culture	16/33 (48)
Tempest	56	Blood culture or transthoracic aspirate	42/56 (75)
Benner	85	Transtracheal aspirate	73/85 (86)
Drew	31	Blood culture	29/32 (94)
Guzzetta	14	Blood culture	5/14 (36)
Gleckman	36	Blood culture	25/28 (89)

Skerett SJ, Clin. Chest. Med 1999; 3: 531-548

“ Identifying the microbial cause of CAP may aid in clinical management …..However, to date, no data document that etiologic diagnostic testing can improve outcome or reduce overall medical costs. ….. This controversy probably will continue until economical, rapid, and accurate diagnotic tests become available.”

Butler JC et al. J. Infect. Dis.2003; 187: 1422

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Utility of Amplification Methods for Virus Detection in CSF

- HSV: PCR was shown to be the reference method

  Lakeman et al, J. Infect. Dis. 1995; 171:857

- Extended to herpes virus group
- Extended to enterovirus detection in cases of meningitis

  Tanel et al., Arch. Pediatr. Adolesc. Med. 1996; 150: 919

  Ahmed A et al, J. Pediatr. 1997; 131: 393

  Van Vliet et al, J. Clin. Microbiol. 1998; 36: 2652

⇒ **Enormous increase of requests for PCR on CSF**

Effective Use of PCR for Diagnosis of CNS Infections

Organism detected	No. (%) of tests with indicated result/no. of tests performed				Total
	Both protein level and leukocyte count normal	Protein level normal, leukocyte count abnormal	Leukocyte count normal, protein level abnormal	Both protein level and leukocyte count abnormal	
Herpesvirus*	0/209 (0)	1/33 (3.0)	5/317 (1.6)	18/173 (10.4)	24/732 (3.3)
T. whippelii	0/56 (0)	0/3 (0)	1/101 (1.0)	0/30 (0)	1/190 (0.5)
B. burgdorferi	0/149 (0)	0/18 (0)	0/215 (0)	0/89 (0)	0/471 (0)

* Including HSV, EBV, VZV, and CMV

Tang et al, Clin. Infect. Dis. 1999; 29: 805-06

Restriction Rules for HSV Detection in CSF

Reference	N° cases / specimens	Criterium
Tang (1999)	24 / 723	WBC > 5 cells / mm^3 and / or > 45 mg/dL protein
⇒ workload reduction 29 %		
Simko (2002)	10 / 406	WBC > 5 cells / mm^3 and / or > 55 mg/dL protein
⇒ workload reduction 38 %		
⇒ increase of positivity rate: 1.9% → 4% 2-fold		

Tang et al, Clin. Infect. Dis. 1999; 29: 803
Simko et al, Clin. Infect. Dis. 2002; 35: 414

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Influence of Prevalence on Predictive Values

for given test : Se = 99%, Sp = 98%

Prevalence	PPV	NPV
1‰	4.9 %	99.99 %
1 ‰	4.7 %	99.99 %
1 %	33.3 %	99.98 %
2 %	50.0 %	99.98 %
3 %	60.0 %	99.97 %
4 %	67.0 %	99.96 %
5 %	72.0 %	99.95 %
10 %	84.0 %	99.89 %
20 %	92.0 %	99.75 %
30 %	95.0 %	99.56 %

Goldberg M, 1990; “L’epidémiologie sans peine”

Evidence based Strategy for the Molecular Detection of MTB

Smear-positive samples only

(1200 cases / 120.000 requests per year / 2 samples per patient / 50% samples smear-pos / 70%: *M. tuberculosis*)

- sens = 95% / spec = 99%
  - PPV = 99.5% or 6 pos results are false pos
  - NPV = 95% or 20 neg results are false neg
- sens = 99% / spec = 99.5 %
  - PPV = 99.7% or 3 pos results are false pos
  - NPV = 99% or 4 neg results are false neg

Evidence Based Molecular Detection of MTB

Stand-alone first-line screening test

- sens = 95% / spec = 99%
  - PPV = 46.9% or 1 out of 2 are false pos
  - NPV = 99.7% or 360 neg results are false neg
- sens = 98% / spec = 99.9 %
  - PPV = 95.2% or 120 pos results are false pos
  - NPV = 99.96% or 47 neg results are false neg

Evidence Based Molecular Detection of MTB

Only highly suspicious smear-negative samples

(prevalence increases from 1 to 10%)

- sens = 75% / spec = 99.75 %
  - PPV = 98.8% or 14.5 positive results are false positive
  - NPV = 97.2% or 300 negative results are false negative

Evidence Based Strategy for the Molecular Detection of MTB

current indications for molecular testing:

- smear-positive samples
- positive liquid cultures

possible additional indications for molecular testing

- smear-negative respiratory and extra-respiratory samples from patients with strong clinical indications

no indication for molecular testing

- first line screening to exclude MTB

Estimated Costs of False Laboratory Diagnosis of Tuberculosis

- **False positive result**

  ⇒ unnecessary TB treatment
  outpatient visits
  contact investigations ⇒ average cost of US$ 10.873
  possible hospitalisation, isolation
  tests and procedures

  Northrup JL et al, Emerg. Infect. Dis. 2002; 8: 1264-1269

- **False negative results**

  ⇒ TB : high morbidity and possible mortality
  deprival: of TB treatment
  contamination of contacts,….

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Treatments for Toxoplasmosis in Pregnancy: COCHRANE REVIEW

- **Objective:** to assess whether or not treating toxoplasmosis in pregnancy reduces the risk of congenital toxoplasmosis

- **Selection criteria:** randomized controlled trials of AB treatment versus no treatment of pregnant women with proven or likely acute Toxoplasma infection, with outcomes in the children reported.

- **Main results:** 3332 papers identified, none met the inclusion criteria

- **Conclusions:** "... we still do not know whether antenatal treatment reduces congenital transmission. Screening is expensive, so we need to evaluate the effects of treatment; and impact of screening programmes, …. these technologies should not be introduced outside the context of a carefully controlled trial.

Peyron F et al, The Cochrane Library, 2002

Screening for *C. trachomatis* : Questions to be Solved

- Is screening effective ?
  - on ↓ of prevalence
  - on ↓ of complications
- Who should be targeted ?
- In which clinical setting should be screened ?
  - systematic screening
  - opportunistic screening
  - selective screening
- What is the preferred method of screening ?
- Is screening feasable and cost-effective ?

Pimenta, Brit. Med. J. 2000; 331:629-631

Prevalence of *C. trachomatis* Infection in General Practice in Antwerp

- **Study population:** 777 sexually active women, age 15-40, visiting their GP
- **Methods:** opportunistic screening by DNA on self-taken vaginal sample

Age	
14 - 17	1/50 (2%)
18 - 22	15/227 (6.6%)
23 - 27	15/260 (5.8%)
28 - 35	8 / 220 (3.6%)
36 - 40	0/30 (0%)

Overall prevalence: 4.96%

Verhoeven V. et al, J. Med. Screening 2003; 10: 14-15

Possible Recommendations for Screening for *Chlamydia trachomatis* in a Sample of Women in General Practice

- All women > 1 partner in the past year

  AND

- All women with two of the following:
  - age 18 - 27 years
  - frequent postcoital bleeding
  - having symptomatic partners
  - no use of contraceptives

⇒ would detect 92.3% of infections and 37.5% of the population would need to be screened

Verhoeven V. et al, J. Med. Screening 2003; 10: 14-15

Selective Screening for *C. trachomatis* in a Sample of Women in General Practice

- Advantages
  - risk profiles are possible (in contrast with other investigations in the general population)
  - evidence based selective screening
    - $\rightarrow \downarrow$ risk false positive
    - $\rightarrow \downarrow$ costs
- Disadvantage
  - selective screening based on behavioural variables: is this feasable for general practitioner ?

Verhoeven V. et al, J. Med. Screening 2003; 10: 14-15

Recommendations and Reports on Screening Tests to Detect *C. trachomatis* Infections.

- Potential adverse consequences caused by false positives: patients should be counceled regarding this potential: routine additional testing to improve predictive value of a positive screening test should be considered if low prevalence.
- Selecting persons for testing who are at high risk can increase the prevalence of infection among the tested persons, thereby reducing screening costs.

CDC, MMWR 2002; 51: 1-27

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Detection of Novel Pathogens in Chronic Diseases: Evidence of Association

- Kochs postulates
- Revision by Rivers
- Hill's criteria and guidelines

Proc. R. Soc. Med, 1965; 58: 295-300

- Fredricks and Relman's reconsiderations

Clin. Microbiol. Rev, 1996; 9: 18-33

Some Chronic Diseases Produced by Novel Microbes

Microbe	Disease
Helicobacter pylori	Peptic ulcer disease, gastric cancer
Tropheryma whippelii	Whipple's disease
Borrelia burgdorferi	Lyme disease
Cyclospora cayatenensis	Diarrhea
Hepatitis C virus	Hepatitis, hepatocellular carcinoma
Human herpesvirus 8 (KSHV)	Kaposi's sarcoma

Novel Pathogens in Chronic Diseases: Evidence of Association

"The most convincing evidence comes from a concordance of evidence arising from different approaches applied by different groups, at different times in different places and under different circumstances

Fredricks MD, Clin. Microbiol. Newsletter, 2002; 24: 41-43

Unexplained Human Diseases: a Role for Infection ?

Disease	infections etiology ??
Kawasaki's disease	HHV-8, parvo B19, STSS, *Chlamydia pneumoniae*
Crohn's disease	*Mycobacterium paratuberculosis*
Sarcoïdosis	*Mycobacterium* spp., HCV
Multiple sclerosis	*Chlamydia pneumoniae,* HHV-6
Diabetes mellitus	Coxsackie virus B4, enteroviruses
Chronic fatigue syndrome	*Mycoplasma*, *Chlamydia*
Coronary Atherosclerosis	CMV, *Helicobacter pylori*, *Chlamydia pneumoniae*

The Role of *C. pneumoniae* in Atherosclerosis is Controversial and Unresolved

- Lack of consistent serologic data
- In vivo results are extremely variable
- Isolation by culture in a very limited number of studies
- Antichlamydial therapy seems not beneficial
- Animal experiments and also in vitro studies tend to support a contributory role for CP infection

197 different surgically removed human atherosclerotic fragments

PCR

2 single and 1 real-time PCR (internal control):

3 different DNA fragments of CP

- a CP PstI fragment
- a CP 53 kDa protein
- VD4 domain of the CP OmpA gene

- CP DNA

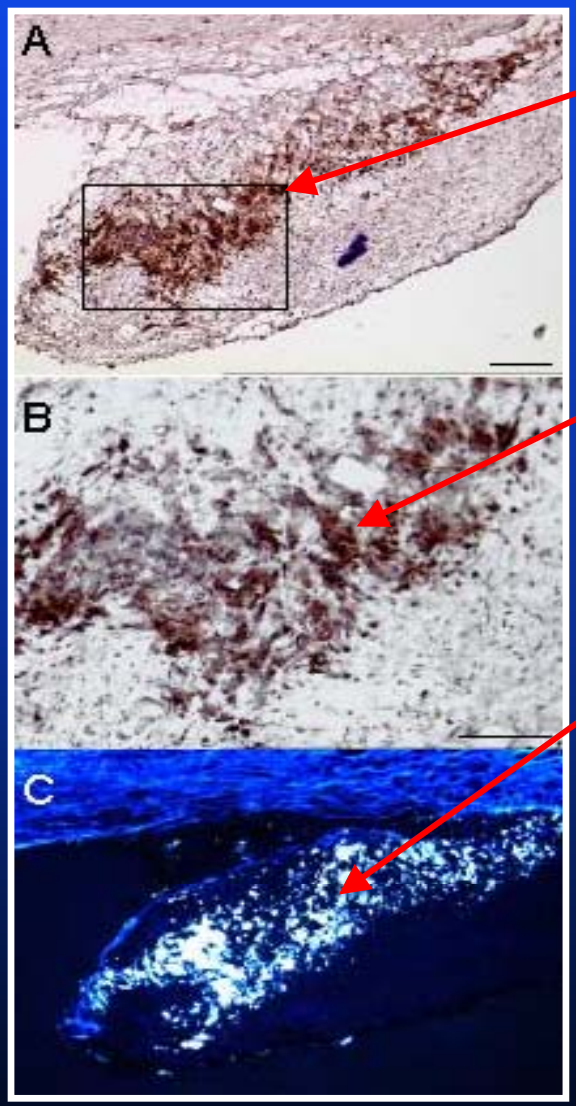
Reanalysis in an independent laboratory, semi-nested PCR

IHC

80/197 fragments IHC with 3 anti- CP MoAbs

- CP membrane protein 79 %, chsp60 16 %, cLPS 11%
- macrophages and SMCs
- aa mammariae showed immunoreactivity

+ CP Ag



Human atherosclerotic plaque positive for CP (RR402)

Close up of the strong positive region

Precise matching of CP immunoreactive staining sites with autofluorescent ceroid

Ceroid: an autofluorescent insoluble lipid pigment, abundantly present in both fatty streaks and advanced lesions within the cytoplasm of lipid-loaden macrophages and foam cell-like SMCs, but also extracellularly in case of necrosis

Conclusion

1. Association CP IgG – atherosclerosis varies with the kind of serological assay. With MIF, no association.
2. No CP DNA detection in human atheroma, nor in the peripheral blood
3. Abundant histological staining with anti-CP MoABs in PCR-negative atheroma
4. Negative WB analyses for CP proteins in strong immunoreactive arteries
5. Autofluorescence under UV light identified the immunoreactive sites in atherosclerotic plaques as ceroid deposits

CP

are not commonly present in atherosclerosis

do not play a direct role in atherogenesis

Evidence Based Microbiological Diagnosis: Conclusions

- "We need less research, better research and research done for the right reasons."

Altman,Brit. Med. J., 1994; 308: 283

⇒ **"We need less diagnostics, better diagnostics and diagnostics done for the right reasons".**

⇒ **There is definitely a need for more communication between the lab and the clinician, and for more interest in identifying optimal strategies for diagnosis.**