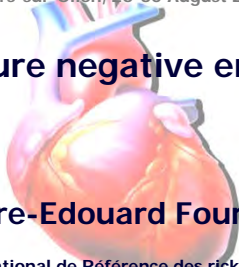


ESCMID Postgraduate Technical Workshop
Intracellular bacteria: from biology to clinic
 Villars-sur-Ollon, 26-30 August 2013

Blood culture negative endocarditis



Pierre-Edouard Fournier

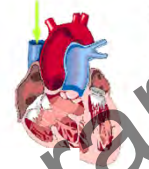
Centre National de Référence des rickettsioses,
 de la fièvre Q et des bartonelloses



Unité de Recherche sur les Maladies Infectieuses Tropicales Emergentes
 URMITE – UMR CNRS 7276, IRD 198, INSERM U1095

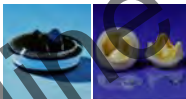
Infective endocarditis

- Colonisation of the endocardium by a microorganism
- Any microorganism
- Valves +++, parietal endocardium



High risk cardiopathies

- Valvular prostheses
- Previous endocarditis
- Congenital cyanogenic cardiopathy
- Ductus arteriosus
- Aortic and mitral leak, interventricular communication
- Aortic coarctation
- Previous cardiac surgery



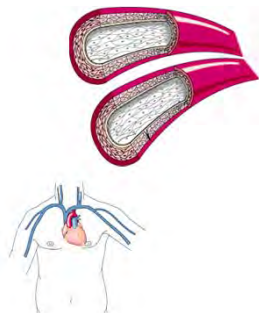
Moderate risk cardiopathies

- Mitral valve prolapse
- Mitral, tricuspid, or pulmonary stenosis, tricuspid leak
- Asymmetric septal hypertrophy
- Aortic bicuspidy
- degenerative valvulopathy in the elderly



Low risk cardiopathies

- Atherosclerosis
- Coronaropathy
- Pace-maker



Infective endocarditis

- ✓ Stable incidence worldwide
- ✓ 15 to 60 cases/million inhabitants/year in the USA and Europe. 145 – 204 cases/million in the elderly
Bashore et al. Curr Probl Cardiol. 2006;31:274-352
- ✓ 0.16 to 5.4 per 1,000 hospital admissions
Sandre and Shafran. Clin Infect Dis. 1996;22:276-86; Martin et al. Clin Infect Dis 1997;24:669-75
- ✓ Changing epidemiology: IVDU, intracardiac devices, nosocomial bacteremias (*Staphylococcus* sp.), elderly (group D streptococci)
Que and Moreillon. Nature Rev. Cardiol. 2011; epub

A severe disease

- ✓ Mortality: 20 to 26% during initial hospitalization, 30% overall

Moreillon and Que. Lancet. 2004;363:139-49

- ✓ Heart surgery required: 25 to 50% of cases

Jault et al. Ann Thorac Surg. 1997;63:1737-41; Larbaletier et al. Circulation. 1992;86:1168-74; Murdoch et al. Arch. Intern. Med. 2009;169:463-473

Diagnosis of infective endocarditis

- ✓ Precise microbiological diagnosis is mandatory to guide therapy
- ✓ Culture was long considered the most important diagnostic tool
- ✓ Highlighted by the weight given to culture in the Duke criteria

Li et al. Clin. Infect. Dis. 2000;30:633-8

Major criteria
Blood culture positive for IE
 Typical microorganisms consistent with IE from 2 separate blood cultures:
 Viridans streptococci, Streptococcus bovis, HACEK group, Staphylococcus aureus, or
 Community-acquired enterococci, in the absence of a primary focus, or
 Microorganisms consistent with IE from persistently positive blood cultures, defined as follows:
 At least 2 positive cultures of blood samples drawn >12 h apart; or
 All of 3 or a majority of <4 separate cultures of blood (with first and last sample drawn >12 h apart)
 Single positive blood culture for *Coxiella burnetii* or antiphase I IgG antibody (titer >1:800)

Minor criteria
 Microbiological evidence: **positive blood culture** that does not meet a major criterion associated with IE

Definite infective endocarditis
 Pathologic criteria
 (1) Microorganisms demonstrated by **culture**, histologic examination of a vegetation,
 a vegetation that has embolized, or an endocardial abscess, or

However

- ✓ Negative blood culture: 2.5 to 31% of patients
 Houptjian and Raoult. Medicine. 2005;84:162-73; Moreillon and Que. Lancet. 2004;363:139-49
- ✓ Valve culture: sensitivity 13 - 26%, specificity 72 - 98%
 Munoz et al. J. Clin. Microbiol. 2008;46:2897-901; Voldstedlund et al. APMS. 2008;116:190-5
- ✓ Should valve biopsies be cultured?
 Munoz et al. J. Clin. Microbiol. 2008;46:2897-901
- ✓ Antibiotics (50-70%), fastidious microorganisms (20-50%)
- ✓ Negative culture => etiological diagnosis delayed => increased risk of valve destruction, septic emboli and death

Is empirical treatment sufficient?

- ✓ Current guidelines = intravenous β -lactam + aminoglycoside
 Badier et al. Circulation. 111:e394-434; Que and Moreillon. Nature Rev. Cardiol. 2011; epub
- ✓ May not treat up to 10% of patients: fastidious bacteria (Q fever, *Brucella* sp., *Legionella* sp., *Mycoplasma* sp., *Tropheryma whippelii*), MDR bacteria, fungi

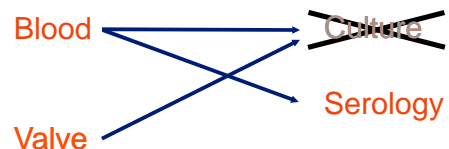
Development of another Diagnostic score for IE

Richet et al. J. Antimicrob. Chemother. 2008;62:1434-40

- ✓ Use of a combination of aspecific clinical symptoms and biological results
- ✓ Criteria independently associated to IE

Variable	Odds ratio	95% CI	P	Score	Proportion of IE	PPV	NPV	Odds ratio
Sex, male	2.5	1.7-3.6	<0.0001	0	10 (4%)	0.04	0.93	baseline 1
Prior valvular damage	8.2	5-13.3	<0.0001	1	30 (6.5%)	0.06	0.96	1.7
Fever	2.1	1.4-3	0.003	2	99 (18%)	0.2	0.93	5.4
Stroke	4.3	2.2-8	<0.0001	3	115 (28%)	0.28	0.82	9.5
Emboli	3.6	1.5-8.6	0.004	4	97 (44%)	0.44	0.72	19
Finger clubbing	2.7	1.1-6.7	0.03	5	43 (67%)	0.67	0.56	50
Splenomegaly	6	1.4-25	0.02	6	10 (83%)	0.83	0.32	123
Leucocytosis	1.6	1.1-2.2	0.01					
Thrombocytopenia	2.3	1.4-3.8	0.002					
Erythrocyte sedimentation rate >50	1.9	1.3-2.7	0.0006					

Diagnostic strategy



Serology

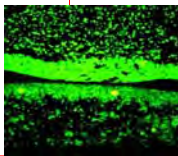
✓ A single serum may identify the causative agent in up to 50% of culture negative cases

✓ *Coxiella burnetii*: IFA: IgG to phase 1 \geq 1:800 (sensitivity 100%, specificity 99.5%), major Duke criterion

Rolain et al. Clin Diagn Lab Immunol. 2003;10:1147-8
Li et al. Clin. Infect. Dis. 2000;30:633-8

✓ *Bartonella* sp.: IFA: IgG > 1:800 (sensitivity 89.5%, specificity 99.6%)

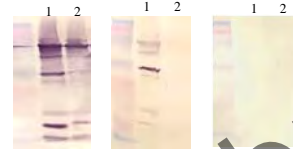
Fournier et al. Clin Diagn Lab Immunol. 2002;9:795-801



Bartonella endocarditis Western blot

✓ Cross reactions among *Bartonella* sp. and with *Coxiella burnetii* or *Chlamydia* => cross adsorption + WB

Non adsorbed Adsorbed Bb Adsorbed Bq



1. *B. quintana* 2. *B. henselae*
Houpikian and Raoult. Clin Diagn Lab Immunol. 2003;10:95-102

✓ Sensitivity 100%, specificity 95%

Serology

✓ *Brucella* sp.: IFA: Ig \geq 1:160

✓ *Legionella pneumophila*: IFA: Ig \geq 1:256

✓ *Mycoplasma pneumoniae*: enzyme immunoassay

✓ *Aspergillus* sp.: ELISA

✓ May be combined into a diagnostic kit

Raoult et al. J Clin Microbiol. 2005;43:5238-42



Microorganisms identified in series of BCNE

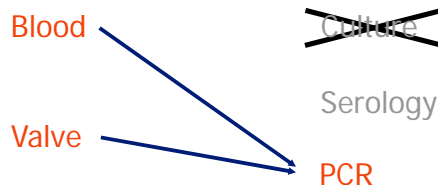
Microorganism	Marseille study (n = 819)	France 2005 (n = 348)	France (n = 88)	Great Britain (n = 63)	Algeria (n = 62)
<i>C. burnetii</i>	33.9	48	7.9	12.7	3.2
<i>Bartonella</i> sp.	12.7	28.4	0	9.5	22.6
<i>Streptococcus</i> sp.	4.6	0	1.1	6.3	3.2
<i>Staphylococcus</i> sp.	1.9	0	3.4	11.1	6.4
<i>F. tularensis</i>	1.8	0.3	0	0	0

✓ Serology panel should be adapted to local epidemiology (*Bartonella* endocarditis <1% in Scandinavia to > 10% in North Africa)

Naber and Erbelli. Int J Antimicrob Agents. 2007;30S:532-6; Brouqui et al. FEMS Immunol Med Microbiol. 2006;47:1-13

<i>Chlamydia</i> sp.	0	0	2.2	0	0
Other bacteria	3.1	1.1	1.1	1.6	1.6
Fungi	1.2	0	0	6.3	1.6
No aetiology	36.5	22.1	82.9	50.8	54.8

Diagnostic strategy



PCR from blood

✓ Broad range assays for bacteria (16S rRNA) or fungi (18S rRNA) => detection and identification

✓ Low sensitivity, specificity may be increased with pre-PCR decontamination by enzymatic digestion

Rothman et al. J Infect Dis. 2002;186:1677-81

✓ LightCycler® SeptiFast: RT-PCR assay, detects 19 bacterial and 6 fungal species

✓ Less sensitive than blood culture (11/50 vs 19/50)

Casalla et al. Eur J Clin Infect Dis. 2009;28:569-573

✓ But may be useful in patients who have taken antibiotics

PCR from valvular biopsies

- ✓ Highest diagnostic yield, fast
- ✓ Widely used method
- ✓ Broad range assays (16S rRNA, 18S rRNA) +/- confirmed by specific assays
- ✓ Sensitivity 41 – 96 %, lower for paraffin-embedded biopsies
- ✓ Specificity 95.3 - 100%

Millar et al. Scand J Infect Dis. 2001;33:673-80; Bosshard et al. Clin Infect Dis. 2003;37:167-72; Breitkopf et al. Circulation. 2003;111:1415-21; Greub et al. Am J Med. 2005;118:230-238; Marin et al. Medicine. 2007;86:195-202

- ✓ Multiplexed PCR (LightCycler® SeptiFast): sensitivity 100%
Fernandez et al. Rev Esp Cardiol. 2010;63:1205-1208)
- ✓ Other molecular detection methods: hybridization probe assays (incl. FISH), NASBA, TMA, LCR, ...

Peters et al. Lancet Infect Dis. 2004;4:751-60

PCR from valvular biopsies

- ✓ Most common agents detected in culture-negative endocarditis:

Brouqui and Raoult. FEMS Immunol Med Microbiol. 2006;47:1-13

- ✓ Fungi
- ✓ Streptococci after antibiotic treatment
- ✓ Fastidious bacteria: *Abitrophia* and other fastidious streptococci, *Bartonella* sp., *C. burnetii*, mycobacteria, *Mycoplasma* sp., *T. whipplei*

- ✓ Should PCR be considered a major Duke criterion?

Millar et al. Scand J Infect Dis. 2001;33:673-80
Lisby et al. Infect Dis Clin north Am. 2002;16:393-412
Bosshard et al. Clin Infect Dis. 2003;37:167-72
Greub et al. Am J Med. 2005;118:230-238
Rice et al. Circulation. 2005;111:1352-4
Syed et al. Prog Cardiovasc Dis. 2007;50:181-97

Pitfalls of PCR

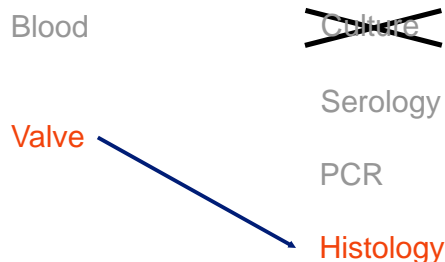
- ✓ DNA persistence in valvular tissues
 - ✓ 10 patients
 - ✓ After completion of antibiotic therapy
 - ✓ 5 months to 7 years (streptococci, *Bartonella* sp.)
 - ✓ No histologic lesion
 - ✓ Past history of IE positive PCR with the same bacterium

Branger and Raoult. J Clin Microbiol. 2003;41:4435-7
Lang et al. Clin Microbiol Infect. 2004;10:579-81
Rovero et al. J Clin Microbiol. 2005;43:163-7

Pitfalls of PCR

- ✓ Many home-made assays => lack of standardization
- ✓ False positive may occur specifically using broad range assays
- ✓ Negative controls are critical
- ✓ Significance of identified agents should be evaluated in the light of epidemio-clinical data
- ✓ Identification of an unusual microorganisms = > confirm by using a second gene target

Diagnostic strategy



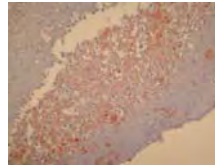
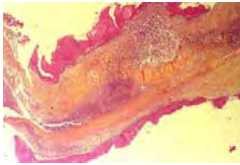
Pathological examination

- ✓ Major Duke criterion
Durack et al. Am J Med. 1994;96:200-22; Li et al. Clin Infect Dis. 2000;30:633-8
- ✓ Gold standard for diagnosis of IE
Morris et al. Clin Infect Dis. 2003;36:697-704; Lepidi et al. Infect Dis Clin North Am. 2002;16:339-61
- ✓ Sensitivity for native valves 73%, for prostheses 42%
- ✓ Specificity 100%
Greub et al. Am J Med. 2005;118:230-8
- ✓ Crucial for blood culture-negative endocarditis
- ✓ Sampling-dependent

Nonspecific staining

Lepidi et al. Infect Dis Clin North Am. 2002;16:339-61

- ✓ Haematoxylin-eosin => inflammatory infiltrate in valvular tissue and vegetations
- ✓ Quantification of PMN > 2% (Anti-CD15 Mabs) => Specificity 98%



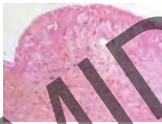
Quantitative analysis of infective endocarditis

Lepidi et al. Infect Dis Clin North Am. 2002;16:339-61

- ✓ Quantification of several histologic parameters:
 - ✓ Intensity and composition of inflammatory infiltrates (neutrophils, macrophages, lymphocytes)
 - ✓ Macrophages (anti-CD68 Ab)
 - ✓ T lymphocytes (anti-CD3 Ab)
 - ✓ Neovascularization (anti-factor VIII Ab)
- ✓ Size of vegetations
- ✓ Quantification of fibrosis and calcifications
- ✓ Quantification of neovascularization

Whipple, *Bartonella*, and Q fever endocarditis

- ✓ Small or absent vegetations
- ✓ Significant fibrosis and calcifications
- ✓ Slight inflammation, composed mainly with mononuclear cells (macrophages and T lymphocytes)
- ✓ Slight neovascularization



Whipplei

Q fever

Bartonella sp.

Special stains



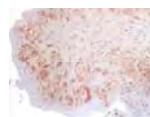
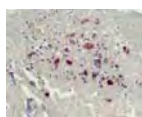
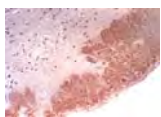
PAS

Warthin-Starry

- ✓ Giemsa → Any bacterium
- ✓ Tissue Gram → Gram-positive bacteria
- ✓ Brown-Hopps → Gram-negative bacteria
- ✓ Brown-Brenn → *T. whipplei*, fungi
- ✓ Periodic acid-Schiff → *Bartonella* sp.
- ✓ Warthin-Starry → Acid fast bacilli
- ✓ Ziehl-Neelsen → *C. burnetii*, *Legionella* sp.
- ✓ Gimenez → Fungi
- ✓ Grocott-Gomori → Fungi

Immunohistochemistry

- ✓ Specific mono- or polyclonal antibodies
- ✓ Immunoperoxidase stain Brouqui et al. Am J Med. 1994;97:451-8
- ✓ Capture-ELISA Thiele et al. Eur J Epidemiol. 1992;8:568-74
- ✓ Immunofluorescence Muhlemann et al. J Clin Microbiol. 1995;33:428-31
McCaull and Williams. Ann NY Acad Sci. 1990;590:136-47



T. whipplei

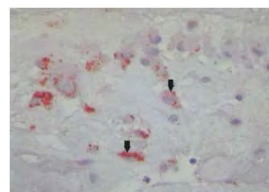
Q fever

Bartonella sp.

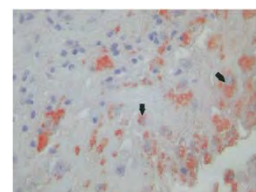
Auto-immunohistochemistry

Lepidi et al. J Infect Dis. 2006;193:1711-7

- ✓ Patients' own antibodies
- ✓ Particularly useful in case of BCNE
- ✓ Sensitivity 80% in streptococcal IE, 100% in *T. whipplei* endocarditis



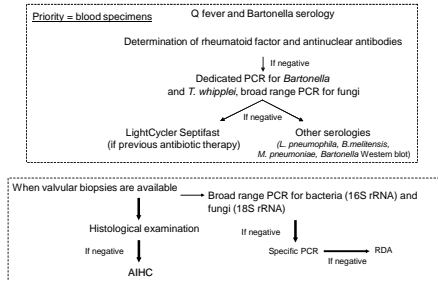
Streptococcal endocarditis



T. whipplei endocarditis

Our diagnostic strategy

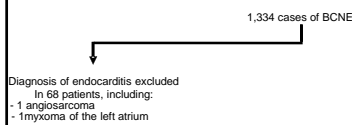
✓ Multi-modal strategy



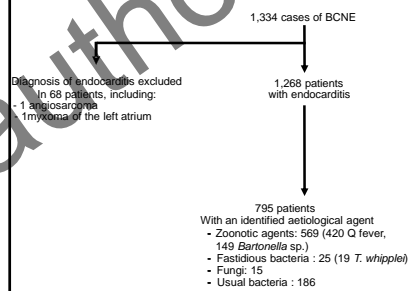
Marseille 2001 - 2011

1,334 cases of BCNE

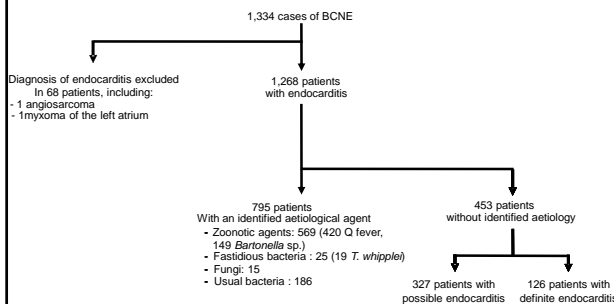
Marseille 2001 - 2011



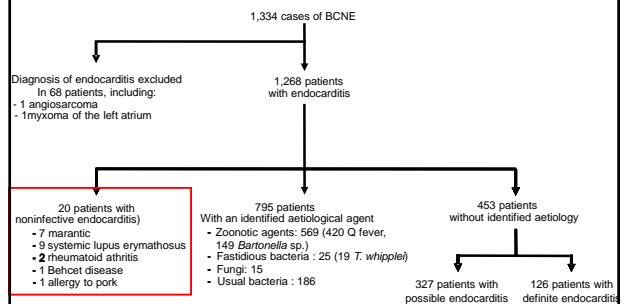
Marseille 2001 - 2011



Marseille 2001 - 2011



Marseille 2001 - 2011



Diagnostic yield of the various methods used

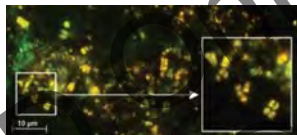
- ✓ Serology => 74.8% of diagnostics
- ✓ PCR => 22.9% additional diagnoses
 - ✓ Blood: only 13.6% positive
 - ✓ Valves: 69.1% positive
- ✓ Culture => no additional diagnosis
- ✓ Auto-immunohistochemistry and differential amplification should be reserved to negative and recurrent cases

Lessons from the Marseille study

- ✓ No *Chlamydia* endocarditis (serological cross-reactions with *Bartonella* sp.)
- ✓ No viral endocarditis (**BUT**: *Coxsackie* B2 endocarditis on an atrio-ventricular patch) Blumental et al. Clin Infect Dis. 2011; 52:710-6
- ✓ Major role of fastidious microorganisms (*C. burnetii*, *Bartonella* sp., *T. whipplei*)
- ✓ Non-infectious aetiologies
- ✓ Should valve culture still be used?

What else? New diagnostic methods

- ✓ Fluorescence in situ hybridization (FISH)
- ✓ Probes for *Staphylococcus* sp., *Streptococcus* sp., *Enterococcus* sp., *Granulicatella* sp., *B. quintana*, *T. whipplei*



- ✓ Valid for both culture-positive and culture-negative endocarditis



Acknowledgements :
Pr. Didier Raoult
Pr. Gilbert Habib
Pr. Frédéric Collard
Dr. Frédérique Gourlet



THANK YOU !