

Research article

## Direct microscopic examination of imprints in patients undergoing cardiac valve replacement

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### Abstract

**Background:** Bacteriological analysis of cardiac valves might be indicated in patients with suspected endocarditis.

**Methods:** We report here a prospective study on fifty-three consecutive patients whose native valves were sent to the bacteriological and pathological laboratories, to investigate the performance of direct microscopic examination of imprints and valve culture.

**Results:** On the basis of a histopathological gold standard to classify the inflammatory valve process, the sensitivity, the specificity, the positive and the negative predictive values of direct microscopic examination of imprints and valve culture were 21%, 100%, 100%, 60%, and 21%, 72%, 38%, 52% respectively. This weak threshold of the direct microscopic examination of imprints could be due to antimicrobial therapy prescribed before cardiac surgery and the fact that the patients came from a tertiary hospital receiving patients with a prolonged history of endocarditis.

**Conclusion:** Clinical context and histopathology are indispensable when analyzing the imprints and valve culture.

### Background

Infective endocarditis accounts for a small fraction of the total number of replaced valves. Histopathologic examination of these tissues removed during surgery is a standard practice in most hospitals. Direct microscopic examination of imprints of the valve is not usually requested. However, bacteriological analysis of cardiac valves might be advised in patients with suspected endocarditis [1]. Laboratories often perform analysis without clinical information. We report here a prospective study

on 53 native valves sent to both bacteriological and pathological laboratories to investigate the performance of direct microscopic examination of imprints (DMEI) and valve culture to confirm and/or find the etiologic agent of acute endocarditis [2,3]. The aim of the study was to determine if routine direct microscopic examination of imprints and the culture of the native valves are informative for confirming and diagnosing endocarditis, using the histopathology as gold standard defining an evolutive inflammatory process.

## Materials and methods

### Valve Study

53 native valves were removed aseptically from patients submitted to cardiac surgery. They were sampled and carried out in sterile containers without medium. The heart valves were manipulated under a laminar flow unit. Portions of the valve showing disease (vegetations, thrombi) were separated into two parts: the first for bacteriological study and the second for pathological study. Before adding formalin, smears of the lesions were made on sterile slides before staining (Gram, May-Grünwald-Giemsa, Gimenez stains) for direct microscopic examination of imprints.

### Bacteriological processing

The valve tissue was ground in 1 ml of brain-heart broth with a mortar and pestle, and then cultured onto 5% sheep blood, chocolate + isovitalax agar and in a paraffined brain-heart broth and Todd-Hewitt broth. The plates were incubated both in aerobic atmosphere and under carbon dioxide atmosphere (5%) for 10 days at 37°C. The broths were kept for 1 month (with subcultures at day 8 and day 30) [2,3]. A chocolate agar was incubated under carbon dioxide atmosphere (5%) for 1 month for *Bartonella sp* detection. A piece of the valve was deep frozen at -80°C. Standard microbiological techniques were performed to identify the bacteria. Results of the May-Grünwald-Giemsa and Gram stains were reported as direct microscopic examination of imprints as follows: rare ( $\leq 1$  per oil immersion [X 1000] field), 1+ (2–9 per oil immersion [X 1000] field), 2+ (10–50 per oil immersion [X 1000] field), 3+ (> 50 per oil immersion [X 1000] field).

### Pathological processing

The tissue specimens were fixed in 10% neutral formalin and embedded in paraffin. Consecutive 5  $\mu$ m sections were stained by hematoxylin-eosin-saffron-astra blue (HES), Gram, Gomori and Periodic Acid Schiff stain methods. The HES allowed us to classify the inflammatory response of the host as acute or chronic/sequellar inflammation [4] considered as the gold standard for evaluating the bacteriological results. The other stains gave information about the presence or absence of microorganisms: this step was considered a direct pathological examination (DPE).

### Patients

53 patients admitted to the cardiology hospital were included in this study over two years (February 1997–February 1999). After the valve laboratory studies, clinical features and other biological parameters were retrospectively collected as follows: blood cultures performed before and during surgery, antimicrobial therapy prescribed, intracellular bacteria serologies (*Bartonella*,

*Coxiella*, *Chlamydiae*, *Legionella*) if the data were available. The number of positive blood cultures was not always available in the results obtained before surgery.

## Results and discussion

During this prospective study over 2 years, 989 native cardiac valves were removed in the cardiology ward. Among these, 53 (5.3%) were sent to both pathology and bacteriology laboratories because endocarditis was suspected during surgery or because the patient was known to have suffered an endocarditis in the previous months. These 53 valves came from 42 men and 11 women (mean age 50.4 years). The valves were classified as follows: 19 mitral valves, 31 aortic valves, 2 tricuspid valves and 1 pulmonary valve. The pathological classification revealed 24 evolutive acute infectious processes considered as true positive and 29 chronic/sequellar inflammatory processes considered as true negative. The detailed results for each patient are reported in Tables 1 (evolutive acute infections) and 2 (chronic/sequellar inflammatory process). Among the 24 with evolutive acute endocarditis, 23 patients were classified with a definite infective endocarditis according to Duke University [5]: two clinical major criteria +/- one pathologic criteria. Among the 29 other patients, 9 were classified with a definite infective endocarditis according to Duke University and 20 as possible infective. On the basis of the histopathological gold standard, the sensitivity, the specificity, the positive predictive value (PPV) and the negative predictive value (NPV) of the DMEI and of the culture are reported on Table 3. The performance of the DPE is also reported in the Table 3. The weak sensitivity of the DMEI could be explained by the thickness of the tissue in contact on the smears performed, and by the antimicrobial therapy decreasing the inoculum. The DPE performed in the tissue is more interesting, giving a sensitivity of 71 % and a specificity of 90%. Similar results were described by Chuard et al. [1] revealing the underfulness of direct examination because a negative result does not eliminate the diagnosis in treated patients. The status of the histopathological lesion seems to be more informative of the evolution of the illness. This could be due to the antimicrobial therapy prescribed before cardiac surgery. In our study, 78% of patients had received antibiotics at the time of cardiac surgery. Prescribed antibiotics were always beta-lactams with or without another antibiotic. If DMEI was positive (patients 2, 4, 5, 8, 9), that did not necessarily indicate antibiotic failure because the bacteria observed were dead.

The valve cultures were negative in 40 patients. In the 33 patients with retrospective documentation, 84 % (28/33) received antibiotics and 60 % (20/33) had positive blood cultures before cardiac surgery. In those with acute endocarditis, 71 % (15/21) had positive blood cul-

**Table 1: Evolutive acute infections: results of DMEI, DPE, culture of valve and clinical features and other biological parameters**

patient	DMEI	Culture of the valve	Blood cultures during the cardiac surgery (3)	Blood cultures before the cardiac surgery	ATB before the surgery (time)	Intra-cellular bacteria serology		
DPE+	1	Neg	<i>S. epidermidis</i>	4 <i>S. epidermidis</i>	/	No	ND	
	2	+++	Neg	4 <i>S. aureus</i>	<i>S. aureus</i>	Yes 4 weeks	ND	
	3	Neg	<i>S. epidermidis</i>	Neg	<i>S. epidermidis</i>			
	4	+++ <sup>1</sup>	Neg	3 <i>S. epidermidis</i>	<i>S. epidermidis</i>	Yes 4 weeks	ND	
	5	+++ <sup>1</sup>	Neg		<i>Streptococcus sp</i> <sup>2</sup>	Yes	ND	
	6	Neg	Neg	Neg	/	Yes	Neg	
	7	Neg	Neg	Neg	<i>Streptococcus sp</i> <sup>2</sup>	Yes 1 month	ND	
	8	+++ <sup>1</sup>	Neg	ND	Neg	Yes 2 days	ND	
	9	+ <sup>1</sup>	Neg	3 <i>S. agalactiae</i>				
	10	Neg	Neg					
	11	Neg	Neg	Neg	<i>S. pneumoniae</i>	Yes 1 month	ND	
	16	Neg	Neg	Neg				
	17	Neg	Neg	Neg	<i>S. bovis</i>	Yes > 1 month	ND	
	18	Neg	Neg	ND	ND	No	ND	
	19	Neg	<i>P. acnes</i>	ND	<i>H. influenzae</i>	Yes	Neg	
	20	Neg	<i>P. aeruginosa</i> + <i>S. hominis</i>	ND	<i>Streptococcus sp</i> <sup>2</sup>	Yes 2 months	ND	
	24	Neg	Neg	Neg	<i>S. bovis</i>	Yes 2 months	ND	
	DPE-	12	Neg	Neg	ND	<i>Campylobacter sp</i>	Yes	ND
		13	Neg	Neg	Neg	<i>S. agalactiae</i>	Yes 1 month	ND
		14	Neg	Neg	Neg	<i>Enterococcus sp</i>	Yes 1 month	ND
15		Neg	<i>S. epidermidis</i>	Neg	/	No	ND	
21		Neg	Neg	Neg	<i>Staphylococcus sp</i>	Yes	ND	
22		Neg	Neg	Neg	<i>S. pneumoniae</i>	Yes 1 month	ND	
23		Neg	Neg	ND	<i>E. faecium</i>	Yes 5 days	ND	

DPE = direct pathological examination; DMEI = direct microscopic examination of imprints; 1 legend in bacteriological processing section, in all cases corresponding to Gram positif cocci; 2 *Streptococcus sp* was identified as belonging to streptococci viridans group; 3 the number before the species means the number of positive blood cultures; the ATB = antibiotic; Neg = negative; ND = not done; retrospective study impossible for patients 3, 9, 10, 16.

tures. The intracellular bacteria serologies (*Bartonella*, *Coxiella*, *Chlamydiae*, *Legionella*) were only performed in 3 patients (patients 6, 19, 26) and were all negative. Patients 2 and 4, with a positive DMEI, were negative for valve culture. The antimicrobial therapy prescribed 4 weeks before surgery corresponding to the last positive blood culture, had probably sterilized the lesions. Valve culture was positive in 13 patients. 12 were evaluable and corresponded to 4 acute inflammatory processes and 8 chronic/sequellar processes. For the 8 true negative patients, except for patients 28 and 29 where bacterial blood cultures were known before surgery, the bacteria found in the valve could probably have been a contamination since the patients did not present evolutive clinical symptoms of endocarditis. As shown by Giladi et al.[6], valve culture is not recommended when acute endocarditis is not suspected. In the 4 endocarditis patients, 2 (patients 1 and 15) with no antimicrobial

treatment had positive valve culture corresponding to the etiologic agent (*Staphylococcus epidermidis*). As described since 1990, the coagulase-negative staphylococci have been more and more implicated in native endocarditis [7]. The 2 other valve cultures were difficult to evaluate. Patient 20 was known to have had a blood culture documented on with a classical etiologic agent (*Streptococcus viridans*) and the mixed valve culture was probably a contamination. In another patient (patient 19) had a positive DMEI (Gram positive rod), a positive valve culture with *Propionibacterium acnes*, but a blood culture yielding *Haemophilus influenzae*. The microbiologist could not conclude anything from these results. *P. acnes* may often be considered as a contaminant, but in a few cases [8] this bacterium has been responsible for true endocarditis on native valves. This weak threshold of the direct microscopic examination of imprints could be due to antimicrobial therapy prescribed before cardiac sur-

**Table 2: Chronic/sequellar processes: results of DMEI, DPE, culture of valve and clinical features and other biological parameters**

	patient	DMEI	Culture of the valve	Blood cultures during the cardiac surgery	Blood cultures before the cardiac surgery	ATB before the surgery (time)	Intra-cellular bacteria serology
DPE+	25	Neg	<i>S. hominis</i>	Neg	/	Yes	ND
	26	Neg	Neg	Neg	<i>S. capitis Acinetobacter sp</i>	Yes	Neg
	27	Neg	Neg	ND			
DPE-	28	Neg	<i>P. acnes</i>	ND	<i>S. agalactiae</i>	Yes > 15 days	ND
	29	Neg	<i>P. acnes</i>	ND	<i>Streptococcus sp</i> <sup>2</sup>	Yes 15 days	ND
	30	Neg	Neg	ND	/	Yes 2 months	ND
	31	Neg	Neg	Neg	/	No	ND
	32	Neg	Neg	Neg	<i>S. aureus</i>	Yes 2 months	ND
	33	Neg	Neg	Neg	<i>S. aureus</i>	Yes 3 days	ND
	34	Neg	Neg	ND	/	Yes 1 month	ND
	35	Neg	Neg	Neg	ND	Yes	ND
	36	Neg	Neg	ND			
	37	Neg	Neg	ND	<i>S. aureus</i>	Yes 1 month	ND
	38	Neg	Neg	ND	ND	No	ND
	39	Neg	<i>P. acnes</i>	ND	Neg	Yes 10 days	ND
	40	Neg	<i>S. epidermidis</i>	ND	/	Yes 2 months	ND
	41	Neg	Neg	ND	<i>Streptococcus sp</i> <sup>2</sup>	Yes	ND
	42	Neg	Neg	ND	/	Yes 2 months	ND
	43	Neg	Neg	Neg	/	Yes	ND
	44	Neg	OPF	Neg	Neg	No	ND
	45	Neg	Neg	Neg	/	Yes	ND
	46	Neg	Neg	ND	/	No	ND
	47	Neg	Neg	ND	ND	Yes 11 days	ND
	48	Neg	Neg	ND	ND	No	ND
	49	Neg	<i>P. acnes</i>	Neg	Neg	Yes 5 days	ND
	50	Neg	Neg	ND			
	51	Neg	Neg	ND			
	52	Neg	<i>Corynebacterium sp</i>	ND	ND	No	ND
	53	Neg	Neg	Neg	<i>S. pneumoniae</i>	Yes > 1 month	ND

DPE = direct pathological examination, DMEI = direct microscopic examination of imprints, 2 *Streptococcus sp* was identified as belonging to streptococci viridans group ATB = antibiotic; Neg = negative, ND = not done, OPF = oro-pharyngeal flora, retrospective study impossible for patients 27, 36, 50, 51.

**Table 3: Performance of the DMEI, DPE and culture of cardiac native valves**

	sensitivity	specificity	PPV	NPV
DMEI	21%	100%	100%	60%
DPE	66%	90%	84%	76%
culture	21%	69%	36%	51%

DMEI = direct microscopic examination of imprints, DPE = direct pathological examination, PPV = positive predictive value, NPV = negative predictive value

gery and the fact that the patients came from a tertiary hospital receiving patients with a prolonged history of endocarditis.

### Conclusion

Although DMEI is a major criterium of the von Reyn classification and is often rapidly available, it was found to be poorly informative in the assessment of endocarditis in this study. The presence of acute inflammation is the crucial point to evaluate the status of the endocarditis process. If in an acute context, no etiologic agent is known, such as fastidious bacteria [9], it is important to perform valve culture. The drawback of this technique is prior antibiotic therapy giving a poor yield. If the valve has undergone a chronic/sequellar process, the culture will not be informative. In this situation or in acute endocarditis, in a patient with no bacterial documentation and/or receiving antimicrobial treatment, it might be interesting to introduce routine molecular techniques [10]. Clinical context and histopathology are essential to validate the results of valve culture [11].

### References

1. Chuard C, Antley CM, Reller LB: **Clinical utility of cardiac valve stain and culture in patients undergoing native valve replacement.** *Arch Pathol Lab Med* 1998, **122**:412-415
2. Mainardi JL, Vandenesch F, Casalta JP, N'Guyen J, Benoit C, Tissot-Dupont H, Maugein J, Weber M, Bouvet A, Etienne J, et al: **Recommandations pour le diagnostic microbiologique et l'étude anatomopathologique des valves cardiaques au cours des endocardites infectieuses.** *Bull Soc Fr Microbiol* 1995, **10**:12-15
3. Gutschik E, the Endocarditis Working Group of the International Society for Chemotherapy: **Microbiological recommendations for the diagnosis and follow-up of infective endocarditis.** *Clin Microbiol Infect* 1998, **4**:S10-S16
4. Loire R, Vissuzaine C, Bruneval P: **Recommandations pour le diagnostic des endocardites. Etude anatomopathologique des valves cardiaques.** *Ann Pathol* 1995, **15**:221-223
5. Durack DT, Lukes AS, Bright DK, Duke endocarditis service: **new criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings.** *Am J Med* 1994, **96**:200-209
6. Giladi M, Szold O, Elami A, Bruckner D, Johnson BL: **Microbiological cultures of heart valves and valve tags are not valuable for patients without infective endocarditis who are undergoing valve replacement.** *Clin Infect Dis* 1997, **24**:884-888
7. Etienne J, Eyken SJ: **Increase in native valve endocarditis caused by coagulase-negative staphylococci: an Anglo-French clinical and microbiological study.** *Br Heart J* 1990, **64**:381-384
8. Günthard H, Hany A, Turina M, Wüst J: **Propionibacterium acnes as a cause of aggressive aortic valve endocarditis and importance of tissue grinding: case report and review.** *J Clin Microbiol* 1994, **32**:3043-3045
9. Brouqui P, Raoult D: **Endocarditis due to rare and fastidious bacteria.** *Clin Microbiol Rev* 2001, **14**:177-207
10. Goldenberger D, Künzli A, Vogt P, Zbinden R, Altweg M: **Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing.** *J Clin Microbiol* 1997, **35**:2733-2739
11. Peterson LR, Hamilton JD, Baron EJ, Tompkins LS, Miller JM, Wilfert CM, Tenover FC, Thomson RB Jr: **Role of clinical microbiology laboratories in the management and control of infectious diseases and delivery of health care.** *Clin Infect Dis* 2001, **32**:605-611

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