Assessment of Treatment Procedures by Means of Bacteriological and Histological Examinations

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The decisive criterion for assessing the value of a therapy directed towards a high antibacterial effect, must be its action on the bacteria. We use biopsies taken at monthly intervals as random sample of the patient's bacterial status. Bacillary counting is made by simultaneous examinations. As it is our aim to obtain reduction of the number of bacteria to zero, any detectable acid-fast material, whether solid, granulated bacilli or bacterial residues, is classified as "positive".

- (1) The best way of determining the quantitative decrease in the number of bacteria during therapy is by counting. We therefore apply the technique after Rees, modified by Krenzien, i.e. mycobacterial counting in the tissue homogenate of formalin-fixed biopsies. The material is stained with carbol fuchsin by heating three times. We differentiate with 4% sulphuric acid for exactly 1 minute and counterstain with malachite green. The lower measuring limit of this method is approximately 10^2 organisms/mg biopsy when the homogenate is not further diluted, and counting is performed according to the aforementioned method.
- (2) A further basis for assessing the therapeutic value is the examination of frozen sections. The sections are prepared from the same biopsy, the other part of which has been homogenized for counting. Cold staining of the section with carbol fuchsin after Pattyn reveals the best results. We differentiate with 0.1% hydrochloric alcohol or with 2-4% sulphuric acid and counterstain with malachite green or methylene blue. This technique allows an exact examination of the entire section with regard to the number of bacteria in nerves, muscles, vessels, etc. and the diagnosis of the histopathological situation. We consider this method as the most important for the decision "negative" or "positive".
- (3) Smears are collected together with the biopsies. In accordance with the Havel technique, blotting paper with carbol fuchsin is placed on the smear and heated only once; differentiation with 0.1% hydrochloric alcohol. This is a very careful technique to harvest a high number of bacteria.

The results obtained by means of these 3 methods are compared with one another for control and final evaluation. As we know from our experimental work with different mycobacteria in different macroorganisms that dead bacteria are also able to cause lengthy granulomatous processes after injection into the tissue and that the bacilli themselves can stay acid-fast, even solidly acid-fast, over a

longer period (granulation is a reactional stage of living mycobacteria), the decrease of mycobacterial mass per mg/biopsy is an important indication to the value and efficacy of a therapeutic measure. The elimination of dead material is therefore included in this criterion for the assessment of a treatment.

This method appears to be satisfactory as far as practical work is concerned. We must, however, bear in mind that mycobacteria not only vary tremendously in shape and structure, but that they are even able to lose their acid-fastness under suitable conditions and nonetheless can multiply and become acid-fast again.

We are certainly aware of the fact that the described method represents no optimal solution. But it quite reliably indicates the trend, despite occasional discrepancies between the results of the different techniques. The examples in the tables may verify this opinion.

Tables 1-3 follow on pages 55-58.

TABLE 1

A case after 2 years of treatment in the status to be discussed as "negative". Patient is not yet classified as "negative". Twenty years of pre-treatment with dapsone

Malta	30 Serial No.		B. J. Name		40 Age	SB009 Pat. No.		
Date	Skin-smear ^a Ma ^b Bo		Biopsy HBI HMI		Dil.	Histology Bact. Remarks		Therapy
1972								
July 21	+					++		
August								
Sept. 2/30		++	5.20 × 10 ⁵	16/100	1 : 2	+++		
		++	1.09 × 10 ⁶	10/100	1 : 2	+++		
Oct. 28	++	++	5.58 × 10 ⁴	0/28	1 : 2			
Nov.	++	+++						
Dec. 9	+	+	6.78 × 10 ⁴	0/67	1 : 2			
1973								
Jan. 8	++	+++	1.48 × 10 ³	0/20	1 : 2	++		
Feb. 3	++	+++	1.34 × 10 ⁴	0/4	1 : 2	+++		
March 3/31	+		1.54 × 10 ⁴	0/8	1 : 2			
	+		1.87 × 10 ⁴	0/13	∅			
April 29			5.27 × 10 ³	0/11	∅			
May 26	+	+	1.28 × 10 ⁵	1/93	∅	+++		
June 23	—		2.60 × 10 ⁴	1/40	∅			
July 21	—					++		
August 18	—		9.68 × 10 ²	1/12	∅ ^d	+		
Sept. 15	+sc		2.96 × 10 ³	1/40	∅ ^d	++		
Oct. 13	++	++	1.08 × 10 ⁵	4/38	1 : 2	+++		
Nov. 10	+	+	1.67 × 10 ³	0/16	∅ ^d	+++		
Dec. 15	+	+	3.10 × 10 ²	0/3	∅ ^d	++		
1974								
Jan. 12	+sc		4.04 × 10 ⁴	0/25	∅	++		
Feb. 9		+	2.45 × 10 ²	0/2	∅ ^d	++		
March 9	—	+	1.61 × 10 ⁴	0/20	∅			
April 6	—	(+)	7.40 × 10 ¹	1/1	∅ ^d	((+))		
May 4	—					((+))		
June 1	—		3.05 × 10 ²	0/1	∅ ^d	+		
July 1/27	—/—		∅	∅	∅ ^d	∅		
August 24	—	+	∅	∅	∅ ^d	∅		
Sept. 21			2.16 × 10 ²	0/1	∅ ^d	((+))		

RAMP
COMB.

^a Malta

^b Borstel

sc scanty

TABLE 2

A case that will require even longer than 2 years. Fourteen years of pre-treatment

Malta		163	R. J.	Age		01864
		Serial No.	Name			Pat. No.
Date	Skin-smear		Biopsy		Histology	
	^a Ma	^b Bo	HBI	HMI	Dil.	Bact.
						Remarks
						Therapy
1972						
July						(+)
August 4		++			1 : 2	+++
Sept. 1/29	++		2.82×10^5	0/67	1 : 2	
	+++		3.28×10^4	0/12	1 : 2	
Oct.						
Nov. 3/21		+++	3.09×10^5	1/104	1 : 2	++
			5.48×10^3	0/25	$\emptyset^d$	
Dec. 19	+	++	1.12×10^5	0/75	1 : 2	
1973						
Jan. 16	++	(+)	3.59×10^3	0/5	1 : 2	+
Feb.						
March 1			7.13×10^3	0/4	1 : 2	
April 3	+		2.94×10^3	0/3	$\emptyset$	
May 2/30	—		5.32×10^2	0/1	$\emptyset$	
	+	((+))	2.52×10^4	0/19	1 : 2	+
June 6	+		1.00×10^4	0/18	$\emptyset$	
July	+	(+)				
August	+sc					
Sept. 18	+		4.68×10^4	0/20	1 : 2	+++
Oct. 30	+		3.05×10^3	0/21	$\emptyset^d$	++
Nov. 27	+		5.48×10^3	0/25	$\emptyset^d$	
Dec.						
1974						
Jan. 8	+		9.38×10^2	0/9	$\emptyset^d$	
Feb. 5	—	$\emptyset$	2.08×10^2	0/3	$\emptyset^d$	(+)
March 5	+	+	1.51×10^4	0/70	$\emptyset^d$	((+))
April 19	—					
May 14	—		1.91×10^3	1/3	$\emptyset^d$	$\emptyset$
June 11	+		$\emptyset$	$\emptyset$	$\emptyset^d$	((+))
July 9	—		6.79×10^2	0/5	$\emptyset^d$	(+)
August 6	+sc	(+)	4.17×10^3	0/12	$\emptyset^d$	(+)
Sept. 3	—		9.62×10^2	0/3	$\emptyset^d$	((+))

RAMP
COMB.

^a Malta
^b Borstel
sc scanty

TABLE 3

A case without pre-treatment, reaching the status to be discussed as "negative" already after 1 year

Malta	Serial No.		P. C. Name	37 Age	28117 Pat. No.		
Date	Skin-smear		Biopsy		Histology		Therapy
	^a Ma	^b Bo	HBI	HMI	Dil.	Bact.	Remarks
1973							
July							
August							
Sept.	+++						
Oct. 15	+		1.12×10^5	1/44	Ø	+	
Nov. 12	++	++				+	
Dec. 10	+		3.48×10^4	5/44	Ø	+	
1974							
Jan. 7	+		1.50×10^4	4/30	Ø ^d	+	
Feb. 4	—		Ø	Ø	Ø ^d	+	
March 4	+	++	1.58×10^5	3/77	1 : 2	+++	
April 1/29	+sc/—	(+)	2.03×10^3	0/20	Ø ^d	((+))	
			2.05×10^3	3/20	Ø ^d	((+))	
May 27	—		4.87×10^2	0/3	Ø ^d	((+))	
June 24	—		2.12×10^2	0/2	Ø ^d	((+))	
July 22	—		5.21×10^2	0/2	Ø ^d	((+))	
August 19	+	((+))	Ø	Ø	Ø ^d	((+))	
Sept. 16	—		5×10^1	0/1	Ø ^d	((+))	

RAMP
COMB.

a Malta

b Borstel

sc scanty

TABLE 4
A typical case with a lower number of bacteria. The reduction of the number of bacteria to zero is reached much more rapidly

Malta	Serial No.		M. I.		29	27634		
			Name		Age	Pat. No.		
Date	Skin-smear		Biopsy		Histology			
	Ma	Bo	HBI	HMI	Dil.	Bact.	Remarks	Therapy
1972								
July								
August								
Sept.								
Oct.								
Nov.								
Dec.								
1973								
Jan.								
Feb.								
March								
April								
May								
June								
July								
August 6		+	5.29×10^3	5/44	$\emptyset^d$	++	} RAMP	
Sept. 24		(+)	1.42×10^2	0/1	$\emptyset^d$			
Oct.								
Nov. 6			$\emptyset$	$\emptyset$	$\emptyset^d$	+		
Dec. 16			$\emptyset$	$\emptyset$	$\emptyset^d$	$\emptyset$		
1974								
Jan. 15			$\emptyset$	$\emptyset$	$\emptyset^d$	$\emptyset$		
Feb.								
March 19		$\emptyset$	$\emptyset$	$\emptyset$	$\emptyset^d$	$\emptyset$		
April								
May								
June								