

THE OPSONIC INDEX IN THE DIAGNOSIS OF MIXED INFECTION IN PULMONARY TUBERCULOSIS *

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The importance of mixed infection in tuberculosis is at present a much-disputed question. Some investigators believe that secondary invasion by pyogenic organisms is responsible for practically all the damage done in "consumption," while others believe that the tubercle bacillus alone is capable of producing all the pathological changes. Between these two extremes of opinion are found those occupying all possible positions in the middle ground. The reason for this great disagreement of opinion among investigators and clinicians is the inefficiency of the methods used in the diagnosis of mixed infection. Practically all of our data on the subject, up to the present time, have been obtained by: (1) animal experimentation; (2) post-mortem histological and bacteriological examination of the lung; (3) blood cultures before and after death; and (4) sputum examination.

The results obtained in animal experimentation are greatly at variance with each other. Sputum inoculated into rabbits and mice has resulted in a rapid septicemia due to the pyogenic organisms present; but Sternberg and Pasteur were able to produce septicemia with normal saliva. Prudden's¹ work in 1894 on the importance of streptococcus in cavity formation in guinea-pigs has been discounted since Marmorek² produced cavities by injecting tubercle bacilli in pure culture together with large quantities of tubercle toxins in 1907.

The results obtained by post-mortem examination of the lung are not reliable because of agonal and post-mortem bacterial invasion. The results of Ravenel³ show many *Bacilli coli*, *Sarcina*, *Proteus vulgares* and other organisms that undoubtedly invade the lung at the time of death or soon after. To just what extent streptococcus is an agonal invader, or at least a terminal infectious agent in these cases is difficult to determine.

Blood-cultures made from the heart's blood immediately after death show the presence of streptococci in a high percentage of cases. Repeated examinations of the blood in the same cases before death were negative.

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1. Prudden, T. M.: New York Med. Jour., 1894, lx, 1.

2. Marmorek, A.: Compt. rend. Soc. d. Biol., 1907, lxii, 123.

3. Ravenel, M.: Rep. Henry Phipps Inst., 1907, iii, 216.

The streptococcus probably invaded the blood-stream in these cases during the agonal period, or soon after (Beco).

Blood-cultures during life have been positive only in far-advanced cases, and in most of the positive cases on record the organism was present as a terminal infection. Many of the early positive results reported are not reliable because sufficient precaution was not taken against contamination. More recently, however, with improved technic, organisms other than the tubercle bacillus have been isolated from the blood of tuberculous patients. In some of these cases the organism was not present as a terminal infection; one of Panichi's⁴ patients did not die until seven months after the blood-culture was made (Panichi⁵).

The results obtained by sputum examination are very unsatisfactory. All the organisms of mixed infection found in the sputum of a patient suffering from pulmonary tuberculosis are found normally in the mouth, pharynx and trachea of the healthy individual. Whether they are present in the healthy lung is a disputed question. Cornet⁶ says that they are not present in the atria and alveoli of the normal lung, and other investigators⁶ say that they are. Because of the great variety of bacteria present normally in the upper air-passages, the sputum examined for organisms of mixed infection has been subjected to washing and the results obtained have varied greatly with the manner and intensity of this procedure. Most authorities advocate washing the fresh sputum in six changes of sterile salt solution. Sörgo⁷ believes that this is insufficient, and says that the sputum should be whipped violently and broken into small bits, from a majority of which both the tubercle bacillus and secondary organisms can be cultivated. His results indicate that mixed infection is not so common as other observers have believed.

Our present methods of diagnosis of mixed infection are uncertain. Hence, a method of serum diagnosis, if reliable, would be of great value. The opsonic index, when introduced, promised to be such a method. Webb⁸ has used it, but considers it too laborious a procedure for routine work. Yet he obtained abnormal indices to pyogenic organisms in cases suffering from pulmonary tuberculosis. He says:

The third method of ascertaining the activity of these secondary organisms, testing the patient's resistance to them as measured by their opsonic indices, is a laborious and unnecessary procedure. In repeated instances patients have been found with a low index to their own staphylococcus and pneumococcus, and in febrile cases they have shown fluctuating indices to these as well as the tubercle bacillus.

4. Panichi: Berl. klin. Wehnschr., 1908, xlv, 1840.

5. Cornet, G.: Tuberculosis, edited by W. B. James, Philadelphia, 1905; W. B. Saunders Company, p. 583.

6. See references given by Norris and Pappenheimer: Jour. Exper. Med., 1905, vi, 48.

7. Sörgo: Ztschr. f. Tuberk., 1904, vi, 382.

8. Webb, G.: In Klebs' Tuberculosis, N. Y., 1909, D. Appleton & Co., p. 594.

Wirths⁹ examined twenty-five cases of tuberculosis. He found no change in the opsonic index to *Diplococcus capsulatus*, *Micrococcus tetragenus catarrhalis*, the meningococcus, *Bacillus pneumoniae*, the pseudodiphtheria bacillus, *Bacillus coli*, or *Bacillus subtilis*, but he found abnormal variation in the opsonic index to the staphylococcus, streptococcus, pneumococcus and influenza bacillus. He considered the normal variation between 0.8 and 1.2. He also found that serums that gave a normal reading on drawing if allowed to stand twenty-four hours gave abnormal readings. He used the usual Wright technic and found the following:

2 abnormal opsonic indices (12 per cent.) out of 17 examined to *Bacillus influenzae*.

2 abnormal opsonic indices (12 per cent.) out of 17 examined to staphylococcus.

18 abnormal opsonic indices (75 per cent.) out of 24 examined to pneumococcus.

6 abnormal opsonic indices (31 per cent.) out of 19 examined to streptococcus.

No change was found in five out of twenty-five cases, or 20 per cent. He found abnormal indices to both pneumococcus and streptococcus in six cases and abnormal indices to both pneumococcus and influenza in two cases, to staphylococcus and pneumococcus in one case. He found no change in the index in patients with hectic fever and he found normal temperatures in cases showing variation in the index to pneumococcus.

I have examined the opsonic index to streptococcus, pneumococcus, and staphylococcus in forty cases of pulmonary tuberculosis. I found the index to all of them between 0.8 and 1.2 in all cases but one and in this case the index to staphylococcus was 0.75. This case was complicated by a rectal sinus. Bacteriological examination of this sinus showed staphylococci. In several of these cases that were far advanced blood-cultures were made and Gram-positive cocci, undoubtedly streptococcus or pneumococcus, obtained. The blood-cultures were confirmed by finding Gram-positive cocci in blood-smears. Leukocyte counts were made in nearly all the cases. The usual Wright technic for the opsonic index was used on serums one or two days old.

In making the blood-cultures, blood (15-25 c.c.) was taken from the cubital vein in a sterile syringe; and 2 c.c. run over agar surfaces in each of two large flasks; and 5-10 c.c. introduced into each of two flasks containing 150 c.c. of litmus milk. Blood-smears were made by puncturing the lobe of the ear of the patient after washing the skin with alcohol and drying with sterile cotton, taking a drop of blood with a sterile loop and spreading between two coverslips. The smears were stained by Gram's method and counterstained with eosin.

9. Wirths, M.: Beitr. z. Klin. d. Tuberk., 1908, xii, 159.

Even in cases of undoubted mixed infection as shown by the fever, the leukocytosis, the positive blood-cultures and the findings of Gram positive organisms in blood-smears, there was no variation in the opsonic index to streptococcus, staphylococcus or pneumococcus.

The results are shown in the accompanying tables:

TABLE 1.—OPSONIC INDEX TO VARIOUS ORGANISMS WITH BLOOD-CULTURE, BLOOD-SMEARS AND LEUKOCYTE COUNT

No.	Classification.	—Opsonic Index to—			Blood-Culture.	Blood-Smears.	Leuko-cytes.
		Pneu-mo-coccus.	Staph-ylo-coccus.	Strep-to-coccus.			
1	Incipient, active.....	.94	.96	.92	8,200
2	Far-advanced, passive...	.96	.97	.94	9,200
3	Advanced, passive.....	.98	.98	1.2	13,200
4	Advanced, active.....	.91	.95	.88	17,000
5	Advanced, active.....	.97	.98	1.1	15,000
6	Advanced, passive.....	.91	.94	.85	14,000
7	Advanced, active.....	.90	.95	1.1
8	Advanced, active.....	.93	.94	1.	11,200
9	Advanced, active.....	.95	.98	.95	17,000
10	Advanced, active.....	.91	.85	.94	34,000
11	Advanced, active.....	.97	.93	.84	22,000
12	Advanced, passive.....	.95	.98	.87	—	+	33,000
13	Advanced, passive.....	.98	.97	.95	17,400
14	Incipient, passive.....	.96	.92	.98	11,000
15	Advanced, active.....	.96	.90	.89
16	Advanced, active.....	.90	1.	.92	18,000
17	Advanced, passive.....	.90	.92	1.2	19,000
18	Advanced, passive.....	.93	.98	.95
19	Advanced, active.....	.93	.95	.91
20	Far-advanced, active....	.96	.94	.85	—	15,000
21	Advanced, active.....	.97	.93	.86	—	35,000
22	Far-advanced90	.87	.94	+	+	17,000
23	Advanced, active.....	.87	.92	.89	15,000
24	Advanced, active.....	.94	.96	.97	6,600
25	Advanced, active.....	.89	.91	.84	+(?)	13,400
26	Incipient, passive.....	.98	1.2	.96	7,000
27	Advanced, active.....	.93	.87	.83	18,000
28	Incipient, passive.....	.97	.96	.90
29	Advanced, active.....	.96	.85	.92	9,800
30	Advanced, passive.....	.96	.96	.98
31	Advanced, passive.....	.94	.96	1.	21,000
32	Advanced, passive.....	.97	.94
33	Far-advanced, active....	.93	.88	...	—	8,400
34	Far-advanced, active....	.90	.93	.91	+	19,000
35	Incipient, passive.....	.98	.94	.89	23,000
36	Advanced, active.....	.96	.95	.84	27,000
37	Advanced, active.....	.96	.97	.84	29,000
38	Advanced, active.....	.96	.87	.83	+	+	43,000
39	Far-advanced, active....	.94	1.	.83	+	+	18,000
40	Advanced, active.....	.98	.94	.85

TABLE 2.—CHARACTERISTICS OF ORGANISMS ISOLATED IN BLOOD-CULTURES

Case 25.—Plain Agar: White scattered colonies; small.

Potato: No growth.

Gelatin at 23°; growth along needle track; slight surface growth.

Litmus Milk: Acid production; fine granular curd; whey not separated.

Bouillon: Single; diplococci; clumps and short chains of Gram-positive cocci.

Case 34.—Plain Agar: No growth.

Potato: No growth.

Gelatin: No growth.

Bouillon: Very slight precipitate (?).

Dextrose Agar: No growth on surface; slight growth along needle track.

Blood-Agar: Clear, dew-like, greenish (?) colonies.

Gram-positive, single, diplococci and short chains, in smears from blood-agar.

Case 39.—Blood-Agar: Few clear colonies of definite greenish color.

Plain Agar: Slight growth, clear colonies.

Potato: No growth.

Litmus Milk: Acid production, coagulation.

Gelatin at 23°: No growth.

Gram-positive cocci in pairs from agar and milk.

Case 38.—Blood-Agar: Small grayish-white scattered colonies.

Plain Agar: Slight dew-like growth.

Potato: No definite growth.

Litmus Milk: Acid production, coagulation, no digestion.

Gelatin: Fine scant growth along needle track.

Gram-positive single and diplococci in milk.

Case 22.—Litmus Milk: Finely granular. Showed Gram-positive diplococci after five days.

Blood-Agar Plate: Small greenish (?) colonies.

No growth on any other media.

Case 12.—Blood-Agar: Profuse white growth.

Plain Agar: White extensive growth.

Potato: White powdery growth.

Litmus Milk: Acid production, coagulation, no digestion.

Bouillon: Profuse growth with precipitate.

Large single, and diplococci and cocci in clumps. Gram-positive.

In all cases, except Case 12, the organisms isolated from the blood show scant growth or none at all on the usual albumin-free culture media. For this reason I believe them to be streptococci and pneumococci rather than staphylococci and that they really came from the blood and were not present as contaminations from the skin or air.

Since this paper was written I have extended the blood-culture work and by certain modifications of technic have demonstrated conclusively that these sparsely growing Gram-positive cocci in pairs and chains come from the blood-stream and are not present in the cultures as contaminations. I have made blood-cultures in seventy-five cases of pulmonary tuberculosis and have found unquestionable streptococci or pneumococci in 45 per cent. of those examined. A report of this work will appear in another paper.

From these results I conclude that, in my hands at least, the opsonic index is not an accurate method of diagnosis of mixed infection in pulmonary tuberculosis.