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Nonanatomic Postmortem Techniques: Postmortem Serology

This report is a preliminary look at efforts to incorporate serological techniques into day-to-day autopsy procedures at the University of Tennessee Medical Units. The efforts arose from a continuing desire to make postmortem examination as sophisticated and complete as possible.

Many diseases have immunological abnormalities which lend themselves to postmortem demonstration. Still others, such as infectious diseases of bacterial, viral, or fungal etiology, may be confirmed by qualitative or quantitative antibody determinations. Cases presented to the autopsy service of the City of Memphis hospitals during the calendar year of 1970 were surveyed. Serological determinations were performed, where appropriate, after provisional anatomic diagnoses were made. In so doing, the service hoped to answer the following questions. (1) Do antibody titers decrease after death, or do they accurately reflect the antemortem state? (2) Are there specific diseases which postmortem serology will aid in diagnosing? (3) Is there a loss of immunoglobulins after death, and does such loss follow a predictable curve which can be used to extrapolate the time of death?

Historical Review

Immunohematology is probably the area of postmortem serology that has received the most attention; consequently, the present study does not include this area of interest. Very few investigators, it seems, have addressed themselves to the status of the immune system in the postmortem state. Spain et al [*1*,*2*] reported results of postmortem electrophoreses performed in cases of sudden death in infancy, as hypogammaglobulinemia had previously been suggested as a contributory cause of death in such cases. Others [*3–5*] have reported postmortem electrophoreses in routine autopsy cases. Robinson and Kellenberger [*6*] attempted to answer the question of whether postmortem plasma protein patterns accurately reflected the antemortem state. They reported that the gamma globulin fraction was the most stable fraction, never decreasing in value but actually showing increased values in some samples obtained at greater intervals after death. Furthermore, the increase in gamma globulin was not likely to be confused with macroglobulinemia, as it consisted of a broad increase in the curve rather than a sharp peak. In summary, they concluded that the correlation of ante and postmortem samples was best if less than 24 hours had elapsed between time of death and autopsy.

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Immunoglobulin analyses of ante and postmortem sera were compared by Brazinsky and Kellenberger [7] in 1970. Paired antemortem and postmortem sera from 32 autopsies were compared for levels of immunoglobulins A, G, and M. Differences of less than 25 percent were found in 22 of 32 IgA and IgG determinations and in 23 of 32 IgM. There was no distinct trend to increase or decrease the postmortem value except in the highest ranges, where there was a slight tendency to increase. No correlation was found between the greater-than-25 percent difference and the number of hours after death or with any specific disease process. If an antemortem value was low, normal, or high, the postmortem value also tended to be low, normal, or high.

Methods

All cases presented to the autopsy service of the City of Memphis hospitals during the calendar year of 1970 were surveyed for this study. Five to ten milliliters of blood was obtained from the right ventricle and centrifuged, and the plasma was separated and frozen until assayed. After appropriate provisional diagnoses were made for each case, the plasma was thawed, and such of the following serological determinations as were indicated were performed.

1. *Pneumotropic viruses:* adenovirus; echo 8, 11; influenza A, B, C; parainfluenza 1, 2, 3; respiratory syncytial; psittacosis; eaton agent
2. *Neutropic viruses:* coxsackie B1-6; coxsackie A1, A4, A7, A8, A9; coxsackie A21 (COE); herpes simplex; mumps (viral and soluble); polio 1-3; echo 4, 9, 16; eastern equine encephalitis; western equine encephalitis; St. Louis equine encephalitis; lymphocytic choriomeningitis
3. *Rickettsiae:* Rocky Mountain spotted fever, rickettsial pox, Q fever
4. *Miscellaneous infectious agents:* toxoplasma, trachoma, measles (rubeola), rubella, varicella, cytomegalic inclusion virus
5. Antistreptolysin O
6. A1 antitrypsin
7. A2 macroglobulin
8. Ceruloplasmin
9. Australia antigen and antibody
10. Cold agglutinins
11. Complement level (serum B, A globulin C'3)
12. C-reactive protein
13. Cryoglobulin
14. *Febrile agglutinins:* typhoid A, B, C, E, D, (O & H); Proteus OX19, OX2, OXK; Brucella abortus; pasturella tularensis; pertussis
15. Haptoglobulin
16. Heterophile agglutination (presumptive) and heterophile (differential)
17. Immunoglobulin levels (IgG, IgA, IgM)
18. Immunoelectrophoresis
19. Leptospira agglutination
20. Rheumatoid factor
21. Streptococcus MG
22. Thyroid autoantibodies
23. VDRL

Results

Comparison of Antemortem and Postmortem Values

In cases where patients had serological determinations made during their hospital course, these same determinations were repeated postmortem. In the great majority of cases, these postmortem values agreed remarkably well with antemortem values. If a difference existed, it usually consisted of a one to two tube dilution decrease in the postmortem value. Two examples of such cases are shown in Tables 1 and 2.

TABLE 1—*Postmortem serology in fatal bronchopneumonia.*

Determination	Antemortem Value	Postmortem Value
Influenza A	≥1:64	≥1:64
Influenza B	1:32	1:16
Influenza C	<1:8	<1:8
Para influenza	<1:8	<1:8
Eaton agent	<1:8	<1:8
Respiratory syncytial virus	1:8	1:8
Adenovirus group	1:32	1:8
Typhoid O	1:20	neg
Typhoid H	1:20	neg
Paratyphoid A	1:80	1:80
Paratyphoid B	neg	neg
OX 19	neg	neg
Brucella abortus	neg	neg

TABLE 2—*Antemortem and postmortem values in Reye's syndrome.*

Determination	Antemortem	Postmortem
ASO	250 T.U.	333 T.U.
Coxsackie B1	1:32	1:32
Coxsackie B2	<1:8	1:16
Coxsackie B3	1:16	1:16
Coxsackie B4	<1:8	<1:8
Coxsackie B5	1:64	1:32
Coxsackie B6	<1:8	<1:8
Echo 4	<1:8	<1:8
Echo 8	1:64	1:32
Echo 9, 11, 16	<1:8	<1:8

Table 1 depicts antemortem and postmortem values from the case of a 59-year-old Negro male who presented three major disease processes. The underlying disease was adenocarcinoma of the prostate which had metastasized to the left kidney. This disease was not manifest clinically and was only detected at autopsy. A second disease was severe acute and chronic cholecystitis. This had led to massive scarring of the common bile duct, with bile retention, jaundice, and resultant cholemic nephrosis.

Because of the debilitation caused by the two aforementioned disease processes, the patient developed the bilateral pneumonia which led to his death. Antemortem sputum cultures were consistently negative and the pneumonia was thought to be of viral etiology. Antemortem viral serological studies were performed, as shown in the table. Postmortem lung cultures were also negative. As seen in Table 1, there is excellent agreement between antemortem and postmortem (performed ten hours after death) values for influenza A, B, C, parainfluenza 1, eaton agent, and the respiratory syncytial virus. The adenovirus

titers showed a two-fold decrease (1:32 to 1:8), while typhoid O and H were 1:20 antemortem and negative postmortem. Parathyroid A and B, OX19, and Brucella abortus titers were identical antemortem and postmortem.

The second case (Table 2) depicts ante and postmortem values in a six-year-old Negro female with Reye's syndrome. This poorly understood entity is characterized by acute fatty infiltration of the liver, kidneys, myocardium, and brain. An abrupt clinical course is seen, sometimes less than 48 hours, with terminal renal and hepatic failure and acute encephalopathy. The etiology is unknown, but the participation of a virus (or group of viruses) has been mentioned, without good substantiation. This case is not mentioned in order to further speculate on the causes of Reye's syndrome but simply to show the correlation between antemortem and postmortem serological values. The antemortem serum was obtained within 24 hours of death, and the postmortem serum was obtained 3.5 hours after death. Most antemortem and postmortem determinations were identical. There was a slight decrease in the coxsackie B5 and echo 8 titers, and a slight increase in the ASO titer. The coxsackie B2 increased from <1:8 to 1:16, at least a two-fold increase. These were the only instances in which titers increased postmortem over their antemortem values. While these may represent laboratory error or postmortem alteration of titers, it may well be that these titers were rising just before death, and a titer on a serum of blood drawn immediately prior to death may have reflected the higher values.

Use of Postmortem Immunoglobulin Determination as an Indicator of Time of Death

If the concentrations of the various immunoglobulins decrease in a predictable manner after death, then theoretically their quantitation could be used to determine the time of death. Obviously, this could be done by constructing a curve for the postmortem decay of immunoglobulins, starting from a known concentration just prior to death. If such decay curves were similar, regardless of the antemortem starting values, then it might be possible to fit a single postmortem value onto an appropriate curve, extrapolate backwards, and get some idea of the time of death. The great pitfall in this line of reasoning is clear, for one would need to know the antemortem value in order to select the proper curve and enter it at the correct point. It is also obvious that knowledge of such antemortem levels will not be likely in forensic cases, where such a determination would have its greatest application. Nevertheless, if it were possible to state that immunoglobulins were decreased to a predictable level by some definite time after death, then quantitation of such immunoglobulins would at least have some limited value.

In this study immunoglobulins were assayed only within the first 24 hours after death, as illustrated in Table 3. The mean values of IgG, A, and M are presented for eleven determinations, along with the range and the expected range for the hospital. Pediatric cases were omitted from this table. As can be seen, immunoglobulin concentrations up to 24 hours after death do not vary greatly from the expected antemortem means. Samples are presently being collected from patients known to be dead for more than 24 hours, but this information is not yet complete. Thus, at the present time postmortem immunoglobulin quantitation cannot be recommended as a means for determining time of death.

TABLE 3—*Postmortem immunoglobulin levels.*

	IgA	IgM	IgG
Mean (11 cases)	298.2	194.5	2527.3
Range	112–568	0–490	960–3560
Expected range	90–450	50–280	800–1800

Use of Specific Postmortem Serological Techniques for Documenting Disease

Several specific serological determinations have been found to be valid up to 24 hours after death, the results correlating well with antemortem values (when available). Among these are the C-reactive protein (CRP), VDRL, antistreptolysin 0, and the rheumatoid factor. The CRP is not too helpful in aiding diagnosis, but the other three mentioned may be valuable confirmatory evidence of specific diseases. A great disappointment has been the inability to find a case with the presence of Australia antigen or antibody. It would seem that present means for detecting these two factors are not sensitive enough.

Determinations of antiviral titers are of obvious value, but care must be taken in their interpretation. A single determination cannot be diagnostic, as a rising titer must be demonstrated. Such is not possible in the case of postmortem serology. However, it is believed that a strongly positive titer accompanying other findings that suggest viral etiology may lend circumstantial evidence to the final diagnosis. This is especially important if viral cultures are not available.

Positive titers for such agents as the Rickettsiae, toxoplasma, CID, and trachoma are also of value in documenting disease, as suitable cultures are not available. We have found the febrile agglutinins to be of limited value. Assays of IgG, A, and M can be useful in specific diseases and are of general interest as additional information in most instances. One example is the situation that exists in establishing the origin of severe infection in neonatal deaths. Theoretically, the presence of IgM or IgA or both should point to intrauterine infection, as these immunoglobulins do not normally cross the placental barrier unless infection is present.

Finally, as this technique is expanded, a greater number of specific serological tests will be used as direct or confirmatory evidence in reaching final diagnoses.

Summary

Serological techniques have been used as an integral part of nonanatomic autopsy procedures at the City of Memphis hospitals during the calendar year 1970. The results indicate that, within reason, postmortem antibody titers do reflect the antemortem state. Occasional changes are seen. These usually reflect a fall in titer, although a few postmortem titers were greater than the available antemortem values.

Postmortem serology has been of value in the diagnosis of some specified infectious diseases when appropriate culture techniques were not available. In addition, confirmatory evidence has been gained in other cases, most notably those with suspected viral etiology.

No appreciable loss of immunoglobulins A, G, and M was found up to 24 hours after death. Assay of samples taken at greater intervals after death is presently in progress, but the findings are too incomplete to serve as a basis for determining time of death.

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