Postmortem Blood Microbiology Using Sterile Autopsy Technique

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NIWAYAMA, G. *Postmortem Blood Microbiology Using Sterile Autopsy Technique*. Tohoku J. exp. Med., 1971, **105** (3), 247-256 — Cervical and cardiac blood samples aseptically taken at different time (within 20 hours after death) from 79 cadavers were microbiologically studied and the results were correlated together with the antemortem blood cultures and antibiotic therapies. In 65 cases (82 per cent), the results of both samples were well correlated with each other regardless of the postmortem time lapse and disease. Further analysis indicates that the culture results of cardiac blood drawn from the additional 10 cadavers (13 per cent) at aseptic autopsy were well correlated with the antemortem microbiological data and culture results of other tissues taken at the same autopsies. This study indicates, therefore, that the time lapse (up to 20 hours after death) does not significantly affect the sterility of the blood specimens.

——— postmortem blood microbiology

The interpretation of microbiological data obtained routinely from postmortem specimens is important. However, the interpretation of this data often presents a problem. It is difficult to determine what role, if any, the cultured organisms played in the etiology, pathogenesis, and therapy of the patient's disease.

The question of agonal and postmortem invasion of body fluids, notably blood, by endogenous bacteria has been of great interest in the past. One can find statements to support the contention that agonal invasion does occur (Richey and Geohring 1918, Schaub *et al.* 1958) and others stating that it does not occur (Canavan and Southard 1914-1915, Giordano and Barnes 1921-1922, Epstein and Kugel 1929, Hunt *et al.* 1929, Kurtin 1958, O'Toole *et al.* 1965).

The author's microbiological study on 420 cases with malignancies included in the previous report (Minckler *et al.* 1966) indicated that no effects of age, sex, or postmortem or postsurgical time interval on the number of sterile cultures of the tissue obtained was noted. This paper concerns an analytical study on the microbiological data of 2 postmortem blood samples aseptically drawn from the cadavers at different times to correlate to the previous investigation.

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MATERIAL AND METHOD

The blood samples were collected from 79 patients who died of various malignancies at Roswell Park Memorial Institute. The first blood sample was aseptically drawn by routine technic from the vessels of the neck about or within 4 hours after death. The cardiac blood was drawn between 4 to 20 hours following death by special aseptic autopsy technic (Niwayama *et al.* 1966 a, b). The autopsy was performed in a specially devised surgical suite which was carefully cleaned and prepared by an autopsy assistant. Only appropriately gowned and masked personnel were allowed to enter the aseptically maintained autopsy area, which was constantly bathed with ultraviolet light. The anterior chest, axillary areas, and abdomen of the cadaver were carefully shaved in the preparation room and the cadaver was moved to the autopsy room. Iodine solution was thoroughly applied on the shaven skin surface. Then, the cadaver was draped with sterile towels and sheets before autopsy dissection. Each tissue including chest wall was dissected with separate sterile autoclaved forceps and scalpel blade (or scissors) to avoid any contamination from one tissue to another. The cardiac blood was collected aseptically into a vacutainer culture bottle (50 ml trypticase soy broth and carbon dioxide) as were the cervical blood samples. These bottles were incubated at 37°C. After 2 days' incubation, 1 ml of vacutainer culture medium was aspirated into 2.5 ml VIM disposable polypropylene syringe and distributed to thioglycollate and trypticase soy agar, along with microscopic examination after Gram staining. The final results were made after at least 5 days' observation. For fungal study a small amount of the patient's blood was inoculated into one tube of Sabouraud's medium at room temperature. All the microbiological examinations were carried out by 2 technologists throughout the study. The tissues removed by aseptic autopsy technic for microbiological study were recorded in the order of removal so that subsequent analytical evaluation for culture results could adequately be made to elucidate a contamination, if any. At the same autopsies both tissues, such as lung, liver, spleen, and marrow, and effusion samples were obtained by similar aseptic technic to detect a possibility of septicemia. In addition, clinical histories, particularly antibiotic therapies and antemortem blood culture results were used for evaluation of the postmortem culture results.

RESULT

As shown in Tables 1 and 2, 79 patients (47 males and 32 females) with malignant diseases were included in this study. About 60 per cent of them were male patients and 73 per cent of them were over 40 years old. The diseases included varieties of carcinomas, leukemia, lymphoma, myeloma, and other sarcomas (melanoma, schwannoma, and liposarcoma).

As shown in Tables 3 and 6, the results of both cervical and cardiac blood samples matched together in 65 cases (82 per cent). In 17 cases with both positive cervical and cardiac blood, additional postmortem tissue samples from the same cases at aseptic autopsies were positive for similar organisms respectively. Among 48 patients whose cervical and cardiac blood samples were sterile, there were 19 patients with sterile antemortem blood culture, but one patient had a single positive antemortem blood culture (*Pseudomonas aeruginosa*). The postmortem microbiological studies on the pericardial and pleural effusions, bone marrow (rib) and lymph nodes from the same patient revealed all sterile results. All the postmortem samples from both effusions and visceral tissues except for lung tissues in this group (48 cases) were sterile. Ten out of 34 samples of the

TABLE 1. *Postmortem blood culture study*

Disease	Sex		Total
	Male	Female	
Carcinoma			
Breat	0	6	6
Gastrointestinal	4	3	7
Genito-urinary	6	0	6
Gynecological	0	5	5
Respiratory	9	2	11
Leukemia	16	8	24
Malignant lymphoma	8	2	10
Multiple myeloma	0	2	2
Malignant melanoma	1	4	5
Malignant schwannoma	1	0	1
retroperitoneum			
Liposarcoma, thigh	1	0	1
Hyperplasia, spleen	1	0	1
Total	47	32	79

TABLE 2. *Postmortem blood culture study*

Age Disease	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	Total
Carcinoma									
Breast	0	0	0	0	3	1	2	0	6
Gastrointestinal	0	0	0	0	2	2	1	2	7
Genito-urinary	0	0	1	2	0	0	2	1	6
Gynecological	0	0	0	0	1	1	2	1	5
Respiratory	0	0	0	1	2	5	1	2	11
Leukemia	3	4	2	0	1	8	1	5	24
Malignant lymphoma	0	2	1	1	3	1	1	1	10
Multiple myeloma	0	0	0	0	0	0	2	0	2
Malignant melanoma	0	0	2	0	2	0	1	0	5
Malignant schwannoma retroperitoneum	0	0	0	1	0	0	0	0	1
Liposarcoma, thigh	0	0	0	1	0	0	0	0	1
Hyperplasia, spleen	0	0	0	0	0	0	1	0	1
Total	3	6	6	6	14	18	14	12	79

lung tissues (34 cases) was non-sterile (*Aerobacter*, *E. coli*, *Diphtheroids*, *Krebsiella*, *Monilia*, *Staphylococcus* and *Streptococcus*). In 11 cases the cervical blood was negative, but the cardiac blood was positive by culture. On the other hand, negative cervical blood and positive cardiac blood were obtained in 3 cases. The correlation of the postmortem study with the antemortem blood cultures and antibiotic therapies was shown in Table 9.

DISCUSSION

Over the years, reports have been quite varied as to the value of results obtained by autopsy blood culture (35³, 38.8⁴, 50¹³, 56¹, 71 per cent ¹¹).

TABLE 3. *Comparative studies on postmortem blood culture: Negative results for cervical blood and heart blood*

	Time interval after death (heart blood drawn)					Total
	Less than 4 hr	Less than 8 hr	Less than 12 hr	Less than 17 hr	Less than 20 hr	
Carcinoma		2	2	1		5
Breast			1	1		2
Gastrointestinal		2	2	1		5
Genito-urinary			4			4
Gynecological		1	2	4		7
Respiratory	1	4	6	3	2	16
Leukemia			2			2
Malignant lymphoma				1	1	2
Multiple myeloma			1	1		3
Malignant melanoma				1		1
Malignant schwannoma	1					1
Liposarcoma, thigh						
Total	2	10	20	13	3	48

TABLE 4. *Comparative studies on postmortem blood culture: Sterile cervical blood and positive heart blood*

	Time interval after death (heart blood drawn)					Total
	Less than 4 hr	Less than 8 hr	Less than 12 hr	Less than 16 hr	Less than 20 hr	
Carcinoma						0
Breast						0
Gastrointestinal					1	3
Genito-urinary					1	1
Gynecological						0
Respiratory			1	1		2
Leukemia				1		2
Malignant lymphoma			1	1		2
Multiple myeloma						0
Malignant melanoma				1		1
Total	0	1	3	5	2	11

Epstein and Kugel (1929) reported that all cardiac blood cultures yielded organisms, regardless of the time of culture.

Richey and Goehring (1918) reported that cultures taken from the cardiac blood at autopsy give positive results more frequently than do those from the arm vein immediately after death. Giordazo and Barnes (1921-1922) mentioned that there was no sustained progressive increase in positive results at successive hours after death. In their series they covered a period of 23 hours after death. Hunt *et al.* (1929) found that the lapse of 12 hours after death did not affect the results of postmortem bacteriological cultures. This observation agreed with the view of Fredette (1916) who believed that cultures obtained a few hours after death were fairly reliable in demonstrating organisms present at the time of death. Kurtin

TABLE 5. *Comparative studies on postmortem blood culture: Positive results for cervical blood and negative results for heart blood*

	Time interval after death (heart blood drawn)					Total
	Less than 4 hr	Less than 8 hr	Less than 12 hr	Less than 16 hr	Less than 20 hr	
Carcinoma						
Breast						0
Gastrointestinal						0
Genito-urinary						0
Gynecological						0
Respiratory						1
Leukemia				1		1
Malignant lymphoma				1		1
Multiple myeloma						0
Malignant melanoma						0
Total	0	1	0	2	0	3

TABLE 6. *Comparative studies on postmortem cultures: Positive results for both cervical and heart blood*

	Time interval after death (heart blood drawn)					Total
	Less than 4 hr	Less than 8 hr	Less than 12 hr	Less than 16 hr	Less than 20 hr	
Carcinoma						
Breast				1		1
Gastrointestinal				2		2
Genito-urinary						0
Gynecological						1
Respiratory			1			1
Leukemia				5		5
Malignant lymphoma		2		1		5
Multiple myeloma						0
Malignant melanoma			1			1
Hyperplasia, spleen			1			1
Total	1	3	3	9	1	17

(1958) concluded that the majority of autopsy cultures should be sterile up to 24 hours after death. He also observed, however, that when the cultures of the cardiac blood at autopsy was positive, a focus of infection in other organs could be identified. The data obtained by Wood *et al.* (1965) support the theory that properly obtained cultures of cardiac blood at autopsy, especially when performed within 15 hours after death, offer a good correlation with anatomic evidence of infection and with antemortem blood culture results. In this time interval there was an 83 per cent positive correlation of culture results with antemortem cultures and anatomic data.

In the present study group, the results of both cervical and cardiac blood samples were well correlated in 65 cases (82 per cent).

In 11 cases (14 per cent) all the cervical blood samples were sterile, but the

TABLE 7. *Comparative study on*

Primary malignancy	Cervical blood	Heart blood	Pleural fluid	Pericard. fluid	Ascites
Respiratory	S	1 E. coli			
Case 1					
Case 2	S	5 Staph. aureus		4 S	1 S
Gastrointestinal	S	1 Streptococcus			
Case 3					
Case 4	S	4 Citrobacter sp.	2 Citrobacter sp.	3 Citrobacter sp.	1 S
Case 5	S	2 Haemophilus sp.			1 S
Genito-urinary	S	4 Pseudomonas aeruginosa	5 S	3 S	2 S
Case 6					
Melanoma	S	6 Clostridium welchii	4 Clostridium welchii	5 Clostridium welchii	2 Clostridium welchii
Case 7					
Leukemia	S	5 Candida albicans	3 S	4 S	1 S
Case 8					
Leukemia	S	1 Pseudomonas aeruginosa			
Case 9					
Lymphoma	S	1 Pseudomonas aeruginosa	Lt.-Pseud. aeruginosa ² Rt.-S ³		
Case 10					
Case 11	S	4 Proteus mirabilis	2 S	3 S	1 S

S-Sterile

cardiac blood samples were non-sterile and demonstrated various types of bacteria as shown in Table 7. The evaluation of these complex data is difficult. In 6 cases (Cases 2, 4, 6, 7, 8, 10) the organisms isolated from the cardiac blood were also recovered from other various tissues obtained at aseptic autopsy. In 2 cases the same organisms were identified before death (blood, sputa and urine in Case 9; sputa in Case 10). Therefore, it seems to be rather reasonable to assume that the positive culture result from cardiac blood indicates sepsis before death in

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Rib	Lung	Liver	Spleen	Lymph node	Remarks
					Mediastinum-S 2
	7 A. aerogenes Staph. aureus	2 S	8 S	6 S	Lung (swab)-S 3
		2 Staph. aureus			Abdomen-S 3
	6 Citrobacter Staph. aureus E. coli				Tail of pancreas-S 5
	3 S				
	8 Pseudomonas-aeruginosa	6 Pseudomonas-aeruginosa	7 Pseudomonas-aeruginosa		Skin-S 1
	9 Clostridium welchii	7 Clostridium welchii			Skin-S 1 Omentum-Clostridium welchii 3 Lung tumor-Clostridium welchii 8
7 S	6 Candida albicans	2 S	8		
3 S				2 S	
4 S	5 S				
	5 S	6 S			

Numbers in the right hand corner indicate order of removal.

these 7 cases although it is not clear why the cervical blood samples were sterile. In Cases 1, 5 and 11, other various specimens obtained at sterile autopsy were totally sterile. Three blood samples and one bone marrow specimen obtained prior to death in Case 11 were also sterile. Therefore, the positive result of cardiac blood at sterile autopsy in these 3 cases was most likely due to postmortem contamination. In Case 3, *Staphylococcus aureus* and *Pneumococcus* were isolated from an abdominal wound prior to death. At autopsy, *Streptococcus* and

TABLE 8. *Comparative study on*

Primary malignancy	Cervical blood	Heart blood	Pleural fluid	Pericard. fluid	Ascites
Leukemia Case 12	Staph. epidermidis	S 2	S 3		S 1
Lymphoma Case 13	Corynebacterium sp.	S 1			
Case 14	Bacteroides sp.	S 5	S 7	S 4	S 1

S-Sterile

TABLE 9. *Comparative studies on antemortem and postmortem blood culture*

	Total (cases)	Antibiotic therapy		Antemortem blood culture			Result of postmortem culture
		None	Done	None	Negative result	Positive result	
Positive cervical and cardiac blood culture	17	2	15	6	7		Aerobacter, E. coli, Proteus, Pseudomonas aeruginosa, Salmonella, Staphylococcus and Streptococcus
Negative cervical and cardiac blood culture	48	19	29	28	19		
Negative cervical and positive cardiac blood culture	11	5	6	6	3	2	E. coli, Candida, Citrobacter, Clostridium Welchii, Hemophilis species, Staphylococcus aureus and Streptococcus
Positive cervical and negative cardiac blood culture	3	2	1	1	2	0	Bacteroides, Corynebacterium species and Staphylococcus epidermidis

Staphylococcus aureus were isolated from the cardiac blood and liver respectively. The reason for these discrepant results was not clear.

In 3 cases (Cases 12, 13, 14), the cervical blood samples were positive, but the tests for cardiac blood were sterile as shown in Table 8. Microbiological study on additional tissue samples from the same patients revealed completely sterile results (Table 8). Under this circumstance, the bacteria identified from the cervical blood samples in these 3 cases, *Corynebacterium*, *Bacteroides species* and *Staphylococcus epidermidis*, are most likely of contamination.

Thus, the culture results of cardiac blood drawn from 75 cadavers (95 per cent)

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Rib	Lung	Liver	Spleen	Lymph node	Remarks
7 S	5 S	6 S	4 S		
			2 S		
8 S	6 S	2 S	3 S		8 Liver tumor

Numbers in the right hand corner indicate order of removal.

at aseptic autopsy were well correlated with the antemortem microbiological data and culture results of other tissues obtained at the same autopsy. False positive culture result was obtained from cardiac blood drawn from 3 cadavers (4 per cent) in spite of strict aseptic technic. Similarly, false positive result was observed from cervical blood in 3 cadavers (4 per cent).

Therefore, positive culture results of the cardiac blood carefully and aseptically drawn from cadavers within 20 hours after death can be interpreted as evidence of antemortem infection. In addition, this study seems to indicate that the time lapse after death (less than 20 hours) does not particularly increase high percentage of contamination in the cardiac blood. These results confirm the findings of earlier studies at Georgetown University (O'Toole *et al.* 1965) and Roswell Park Memorial Institute (Minckler *et al.* 1966).

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