Possible Use of Frequency-Pulse-Modulated Electron Capture Gas-Liquid Chromatography to Identify Septic and Aseptic Causes of Pleural Effusions

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Frequency-pulse-modulated electron capture gas-liquid chromatography was used in conjunction with appropriate derivatization procedures to obtain chromatograms from extracts of pleural effusions. These chromatograms were used to rapidly classify the various types of pleural effusions. With this method we have been able to distinguish among a limited number of effusions caused by congestive heart failure, *Mycobacterium tuberculosis*, and some other types of bacterial empyemas and pleural effusions.

Accumulation of fluid or pus (empyema) within the pleural cavity requires that the cause of the effusion be established and that specific treatment, if available, be applied (11). Rapid treatment and removal of inflammatory exudates prevents chronic empyema and the development of fibrosis in the pleural space with subsequent loss of pulmonary function.

The most common causes of inflammatory effusions are tuberculosis, bronchogenic carcinoma, bacterial pneumonia, pulmonary infarction, metastatic tumors, and trauma. The most common causes of noninflammatory effusion are congestive heart failure, cirrhosis, and nephrotic syndrome. Among the less common causes of pleural effusions are systemic lupus erythematosus and pancreatitis.

Empyema is generally caused by bacterial infections (11). A diagnostic procedure which would provide a rapid means (2.5 to 3 h) of differentiating the various causative agents would be a definite asset to better therapy. In this study we used frequency-pulse-modulated electron capture gas-liquid chromatography (FPMEC-GLC) to study pleural effusions and empyemas in the hope that this procedure would provide a powerful analytical tool for rapid diagnosis.

MATERIALS AND METHODS

Sample, source, and handling. A total of 32 samples of pleural fluids from 32 different patients were collected by us at the Blue Ridge Sanatorium in Charlottesville, Va., and at Grady Memorial Hospital in Atlanta, Ga. The causes of the pleural effusions

were determined by the standard laboratory methods employed in the two hospitals. After a portion of the pleural fluid was taken for routine laboratory analysis, the remainder of the sample was assigned a code number and frozen for future FPMEC-GLC analysis.

Derivatization procedures. A 2-ml sample of pleural fluid was extracted at pH 2 with 6% ethanol in chloroform for subsequent esterification of the extracted components with trichloroethanol (TCE) as described previously (1). As a modification we now recommend 6 drops of heptafluorobutyric anhydride (HFBA) instead of 3 drops to assure derivatization with TCE when 6% ethanol in chloroform is used as the extracting solvent. The derivatized sample was placed in xylene, and the excess TCE was removed by heating and evaporating the mixture under a stream of air (1). Care must be taken to remove almost all of the ethanol before derivatization with TCE, but the sample must not be taken to complete dryness either before or after derivatization. A second sample was extracted at pH 2 with ethyl ether for recovery of alcohols and hydroxy acids and subsequent esterification and diesterification with HFBA and HFBA-ethanol (HFBA-EtOH) as described previously (4). The residual aqueous layers from the ethanol-chloroform and ethyl ether extractions were then made basic for extraction of amines and subsequent derivatization with HFBA as described previously (4).

Analytical procedures. The samples derivatized with TCE, HFBA, and HFBA-EtOH were analyzed on Perkin-Elmer gas chromatographs (models 900 and 3920). The instruments were equipped with FPMEC detectors and glass columns (0.32-cm ID by 7.6 m long) which were packed with 3% OV-1 on Chromosorb W (80 to 100 mesh). For TCE esters, the instruments were programmed from 110 to 245°C at 4°C/min and then held at 245°C for 48 min. The electrometer settings, argon-methane flow rates, and recorder parameters were described previously (4). A 2-μl quantity of sample was injected for each analysis.

A Perkin-Elmer PEP-1 computer equipped with a 12K memory and MS-2 revision C software was used to aid in data processing and quality control. The

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computer interfaces were connected to the attenuated pins of the electrometers. Residual TCE was used as a computer reference peak (air peak) for TCE esters, and residual chloroform was used as the reference (air peak) for HFBA and HFBA-EtOH derivatives. The air peaks were used to obtain relative retention times. These retention times were determined from time measurements beginning with the air peak and ending at the crest of each peak.

RESULTS

A total of 14 samples of pleural fluids taken from both treated and nontreated patients with pleural effusions due to infection with *Mycobacterium tuberculosis* were analyzed. The clinical data and FPMEC-GLC groupings are given in Table 1. Typical patterns obtained from the analysis of TCE derivatives of pH 2 chloroform-ethanol extractions are shown in Fig. 1 and 2. Chromatogram A (Fig. 1) was obtained from the pleural effusion of an untreated patient with acute infection caused by *M. tuberculosis*. Both the pleural fluid and sputum from the patients represented by the profile in chromatogram A were culture positive for *M. tuberculosis*. Chromatogram B (Fig. 1) is the type of profile obtained with pleural fluids taken from a treated patient with pleural effusion due to *M. tuberculosis*. Observe that almost all peaks present in chromatogram A are missing in chromatogram B. The patient represented in chromatogram B had been treated with isoniazid, ethambutol,

and streptomycin before the pleural fluid was removed. Other treated patients had similar profiles (Table 1). Chromatogram C (Fig. 1) is representative of the type of profile obtained from the analysis of TCE derivatives of pleural effusions caused by congestive heart failure. Observe that the profile in chromatogram C differs quan-

FIG. 1. FPMEC-GLC chromatograms of TCE derivatives prepared from 6% ethanol-chloroform extractions.

TABLE 1. Clinical information and FPMEC-GLC grouping of effusions caused by *M. tuberculosis*

CDC no.	Final diagnosis from chart (culture-proven pulmonary tuberculosis)	FPMEC-GLC grouping	Chart review findings							
			Protein	Sugar	Sp gr	LDH ^a	Cell count			
							Total	% Polys	% Lymphocytes	% Others
CA 537	Sepsis, <i>M. tuberculosis</i>	IV	NA ^b	37	1.017	ND ^c	600	1	98	1
CA 539	Sepsis, <i>M. tuberculosis</i>	IV	5.6	74	1.025	ND	2,750	ND	95	ND
CA 535	Sepsis, <i>M. tuberculosis</i>	IV	5.0	47	1.019	285	4,440	1	99	0
CA 810	Sepsis, <i>M. tuberculosis</i>	IV	5.8	ND	1.022	ND	150	98	2	0
CA 813	Sepsis, <i>M. tuberculosis</i>	IV	5.3	ND	1.018	ND	609	2	98	0
CA 540	Sepsis, <i>M. tuberculosis</i> , Rx for 1 day ^d		4.4	2	1.020	ND	2,000	69	24	ND
CA 812	Sepsis, <i>M. tuberculosis</i>	IV	5.6	76	1.026	275	152	1	99	0
CA 538	Sepsis, <i>M. tuberculosis</i>	IV	3.7	83	1.027	300	1,625	16	84	0
CA 534	Sepsis, <i>M. tuberculosis</i>	IV	ND	65	1.020	ND	1,760	1	99	0
CA 536	Sepsis, <i>M. tuberculosis</i> , Rx for 18 days		4.9	80	ND	565	14,200	ND	ND	ND
CA 814	Sepsis, <i>M. tuberculosis</i> , Rx for several months	V	5.1	3	ND	3,270	9,800	89	11	0
CA 541	Sepsis, <i>M. tuberculosis</i> , Rx	V	4.3	0	1.019	615	200	20	80	0
CA 811	Sepsis, <i>M. tuberculosis</i> , Rx for 2 months	V	7	92	1.020	412	1,350	44	56	0
CA 815	Sepsis, <i>M. tuberculosis</i> , Rx	V	NA	NA	NA	NA	NA	NA	NA	NA

^a LDH, Lactate dehydrogenase.

^b NA, Not available.

^c ND, No data.

^d Rx, Specific treatment for *M. tuberculosis*.

tatively from the profile in chromatogram A in all peaks and differs qualitatively in the absence of peaks 2, 3, 4, and 11. Peak 16, although not labeled in chromatogram C, was present. Chromatogram B differs from both A and C in that it is almost devoid of peaks.

Table 2 shows a group of specimens, along

FIG. 2. FPMGC chromatograms of TCE derivatives prepared from 6% ethanol-chloroform extractions.

with their clinical data, which were first diagnosed as pleural effusions caused by congestive heart failure. The pleural fluids were coded and grouped by FPMGC-GLC; then a comparison was made between the original diagnosis, the FPMGC-GLC grouping, and the final diagnosis made from clinical data. It is evident from Table 2 that the FPMGC-GLC groupings correlate well with the final diagnosis made from an independent review of the clinical data.

Figure 2 shows additional profiles of TCE-derivatized extracts of pleural fluids taken from patients with pleural effusions caused by infection with *M. tuberculosis*. These profiles are presented to show the degree of reproducibility of chromatograms obtained from the study of these well-documented cases. Qualitatively, the reproducibility was good between CA 537 (Fig. 1, chromatogram A) and CA 534, CA 535, and CA 540 (Fig. 2, chromatograms A, B, and C). Quantitatively, the reproducibility was good between peaks 4, 10, 11, 13, 14, 15, 16, and 20. Peaks 1, 2, 3, and 5 only varied between 1/3 and full scale. In chromatogram C (Fig. 2), peak 1 is full scale and peak 3 is so low that we did not give it a label; however, the increase in peak 1 may be due to the fact that therapy had been initiated before analysis of the sample. In chromatogram D (Fig. 2) trace amounts of some peaks found in profiles obtained for acute cases are still present, but they are markedly reduced in quantity in comparison with those obtained for the acute cases. Some peaks are missing altogether.

Figure 3 and Table 3 show additional profiles and FPMGC-GLC groups obtained from the

TABLE 2. Comparison of a group of pleural effusions originally diagnosed as chronic heart failure with final diagnosis from chart review and FPMGC-GLC grouping

CDC no.	Final diagnosis from chart	FPMGC-GLC grouping before chart review	Diagnosis submitted with specimen	Chart review findings							
				Protein	Sugar	Sp gr	LDH ^a	Cell count			
								Total	% Polys	% Lymphocytes	% Others
CA 762	NA ^b	I	CHF ^c	NA	NA	NA	NA	NA	NA	NA	NA
CA 763	Sepsis from <i>S. aureus</i> pneumonia, Rx ^d	I	CHF	1.6	ND ^e	ND	ND	350	70	30	0
CA 761	CHF	II	CHF	ND	ND	ND	ND	ND	ND	ND	ND
CA 769	CHF	II	CHF	1.8	178	ND	ND	370	6	60	34
CA 566	CHF	II	CHF	2.0	ND	ND	74	1,290	11	72	17
CA 565	CHF	II	CHF	1.2	150	ND	ND	166	34	2	64
CA 766	Sepsis from a urinary tract infection	III	CHF	1.1	103	ND	ND	ND	ND	ND	ND
CA 764	Sepsis from lobar pneumonia	III	CHF	2.9	123	ND	>1,000	ND	Clotted	Clotted	ND
CA 567	Sepsis from <i>S. pneumoniae</i> , Rx	III	CHF	ND	120	1.014	ND	460	3	55	42

^a LDH, Lactate dehydrogenase.

^b NA, Not available.

^c CHF, Chronic heart failure.

^d Rx, Treatment with antibiotics.

^e ND, No data.

FIG. 3. FPMEC-GLC chromatograms of TCE derivatives prepared from 6% ethanol-chloroform extractions. The anaerobe isolated in chromatogram C was identified as *B. fragilis*.

TABLE 3. FPMEC-GLC grouping and chart diagnosis for miscellaneous causes of pleural effusions

CDC specimen no.	Cause of pleural effusion	FPMEC-GLC group
CA 770	Cancer	VI
CA 765	Cancer	VII
CA 768	Cancer	VIII
CA 568	Uremic	VIII
CA 572	Pancreatitis	IX
CA 589	Pancreatitis	V ^a
CA 571	Systemic lupus erythematosus	X
CA 771	Sepsis, <i>Klebsiella</i>	XI
CA 573	Anaerobic empyema	XII

^a FPMEC-GLC analysis showed the pleural fluid to be devoid of detectable amines, acids, and alcohol.

analysis of derivatized extracts of pleural fluids. Chromatogram A is the profile obtained from a TCE-derivatized pleural effusion caused by systemic lupus erythematosus. Chromatogram B is the type of profile obtained from a pleural effusion after pancreatitis. Note that profile B differs from A, C, and D both quantitatively and qualitatively in the first 60 min of the chromatogram. Chromatogram C is the TCE derivative profile obtained from a patient with effusion due to

breast cancer and empyema from which an anaerobe identified as *Bacteroides fragilis* was cultured. Note the abundance of short-chain acids (peaks 1 through 6). Profile C differs from profile A mainly in peaks 1, 2, 5, 8, and 9. The TCE derivatives in chromatogram D represent an empyema caused by *Staphylococcus aureus*. Profile D differs from profile C in peaks 2, 4, 5, and 7 and from chromatogram A in peaks 1, 4, 6, 7, 8, and 9. Chromatogram C differed from chromatograms A, B, and C (Fig. 1 and 2) quantitatively in peak 3 and qualitatively in the presence of peak 6.

Figure 4 shows chromatograms obtained by FPMEC-GLC analysis of pH 2 ethyl ether extracts derivatized with HFBA-EtOH. Chromatogram A is the type of profile obtained from patients with untreated pleural effusions caused by *M. tuberculosis*, and chromatogram B is representative of the type of profile obtained after drug therapy. As found with the TCE derivatives, most of the peaks present in the sample from an untreated patient are missing in the sample from a treated patient. Lactic acid is still present, but in a reduced quantity. Chromato-

FIG. 4. FPMEC-GLC chromatograms of HFBA-EtOH derivatives prepared from ethyl extractions. α -hyd. but. acid, α -Hydroxybutyric acid.

gram C was obtained by analysis of fluid taken from a patient with breast cancer. Observe that profile C differs from both A and B in the presence of high-boiling compounds (eluting after 25 min). Chromatogram C also differs from chromatogram D, which is representative of patients with congestive heart failure, in the number of peaks detected in the high boiling range (33 to 36 and 42 to 58 min). The amount of lactic acid was approximately the same in chromatograms B, C, and D.

Figure 5 shows chromatograms obtained by analysis of HFBA-derivatized pH 10 chloroform extracts of pleural fluids. Chromatogram A represents a patient with an untreated pleural effusion caused by *M. tuberculosis*, and chromatogram B is representative of treated cases. Observe that, as found in the pH 2 extractions (Fig. 1, chromatogram B, and Fig. 2, chromatogram D), the basic extractable compounds have changed with treatment, as indicated by the disappearance of most of the peaks seen in chromatogram A. Chromatograms C and D are the basic types of extractable profiles obtained from effusions due to involvement of the pleura by breast cancer (C) and to metastasis to the pleura (D). Observe that the profile obtained from the

endometrial carcinoma (D) differs from that obtained from effusion due to breast cancer (C) and that both profiles C and D differ from those obtained for tuberculous effusions from non-treated and treated patients (profiles A and B).

DISCUSSION

All of the chromatograms with similar profiles were assigned to groups by a researcher having no prior knowledge of the diagnosis. When these groupings were compared with the tentative diagnoses submitted with the specimens, in two instances the profile obtained by FPMEC-GLC indicated an infectious type of pattern but the effusions were diagnosed as being due to chronic congestive heart failure. A check of data recorded in the histories of the patients showed that the tentative diagnosis originally given to us was in error and that the effusions were, in fact, due to active infections (Table 2).

A representative number of pleural effusions (14 cases) caused by *M. tuberculosis* was studied along with a smaller number (4 cases) caused by chronic congestive heart failure. Tracings shown in Fig. 1 through 5, other than those representing pleural effusions due to nontreated and treated cases of *M. tuberculosis* and chronic congestive heart failure, represent data obtained from analysis of one to three specimens taken from different patients with effusions due to the causes indicated. Judging from these profiles with fewer numbers of samples, we conclude (Tables 1, 2, and 3) that in many instances the FPMEC-GLC patterns obtained from different types of effusions differ and may be of diagnostic value. The degree of reliability of the FPMEC-GLC profiles for making diagnoses can only be established by continued analysis of additional pleural fluid samples accompanied by accurate, well-documented patient histories. Once reliability is established the FPMEC-GLC procedures could offer physicians, hospitals, and clinical laboratories a valuable new tool for use in the rapid diagnosis of pleural effusions.

Data already published (1-3, 5, 7, 8-10, 12) and presented here give strong evidence that FPMEC-GLC might be used as an effective aid in the following areas: (i) diagnosis and study of causative agents of arthritis; (ii) diagnosis of tuberculous and cryptococcal meningitis; (iii) possible diagnosis of certain viral meningitides; (iv) diagnosis of diseases or conditions causing pleural effusions; and (v) *in vitro* identification of microorganisms. In addition to their value for diagnostic purposes, the compounds detected in the FPMEC-GLC profiles are, in some instances, important from the standpoint of their physiological effect on the host. For example, nitros-

FIG. 5. FPMEC-GLC chromatograms of HFBA derivatives prepared from chloroform extractions.

amines, which microorganisms are reported to produce in vivo and in vitro (6, 13), are potent carcinogens. A recently discovered indolic amine (10) found in the cerebrospinal fluids of patients with tuberculous meningitis may also be of clinical importance. The structure of this compound resembles that of certain neurotransmitter amines as well as melatonin. Therefore, it may be of pathophysiological significance in the disease manifestations seen. Treatments might be devised that could relieve the effects of such compounds even before traditional therapy was effective against the causative agent.

In the near future, hospitals and clinical laboratories will begin to investigate the diagnostic potential of FPMEC-GLC. During this period many new applications should be found for the system, and on the basis of instrumentation now available it could be further automated and computerized. The rate of future development and application depends on the support of the scientific community toward its development and on the awareness by teachers, physicians, scientists, and technicians of the new technology and its possible applications.

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