Capnocytophaga sputigena Bacteremia Associated with Acute Leukemia

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Abstract

During a three-year period, *Capnocytophaga sputigena* bacteremia occurred in three patients with acute leukemia receiving induction therapy on a hematology ward. Oral pathology such as periodontitis or severe mucositis was considered to be the most likely source of bacteremia. All three blood culture isolates were identified as that species by deoxyribonucleic acid (DNA) homology studies. Because of the phenotypical similarity of *Capnocytophaga* species, it is difficult to differentiate them by conventional bacteriological methods. All three isolates were susceptible to antibiotics active against most anaerobes. However, production of β -lactamase was found in two isolates, one of which proved resistant to both piperacillin and ceftazidime. Therefore, the empiric use of imipenem or clindamycin may be justified in febrile granulocytopenic patients with cancer who develop significant oral lesions.

Introduction

The genus *Capnocytophaga* is part of the normal oral microflora^{1)~3)}. The organisms have been incriminated as major pathogens in oral pathology such as periodontitis or mucositis^{2)~5)}. *Capnocytophaga* bacteremia has recently been reported to occur with increasing frequency in granulocytopenic patients, particularly in those with acute leukemia receiving intensive chemotherapy or in those undergoing bone marrow transplantation^{4,5)}. Indeed, some of the recent bacteremias have originated from the oropharynx, although the gastrointestinal tract has been the most common source of bacteremia^{4,6)~8)}.

We describe here three cases of *Capnocytophaga sputigena* bacteremia associated with acute leukemia, focusing on the role of oral pathology as the portal of entry and on the genomic speciation of blood culture isolates as well as on the clinical significance of their β -lactamase-producing ability.

Outline of Cases

During a three-year period, 1989–1991, we experienced three cases of *C. sputigena* bacteremia in a similar clinical setting at the Third Department of Medicine, Kanazawa University Hospital (Table 1). Three women were admitted for evaluation and treatment of acute leukemia. They became febrile during

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Table 1 Clinical summary of patients with *C. sputigena* bacteremia

Patient	M.M.	Y.K.	J.O.
Age/Sex	36/F	37/F	28/F
Underlying illness	APL	AML	ALL
Time from start of the last course of anti-leukemic therapy to onset	18 days	24 days	21 days
Granulocyte count at onset	110/ μ l	0/ μ l	0/ μ l
Subsequent changes in granulocyte count* ¹	Increased	Increased	Increased
Hematologic response to antileukemic therapy	CR	CR	CR
Primary infectious focus	Periodontitis	Periodontitis	Oropharyngeal mucositis
Temperature at onset	38.9°C	38.9°C	38.5°C
Complications			
Bacteremic shock	—	—	—
DIC	—	—	—
Serum protein level at onset	6.5g/dl	6.6g/dl	5.0g/dl
Prior antibiotics* ²	FOM+GM	CMZ+GM	PIPC+CAZ+GM
Antibiotic therapy	IPM/CS+GM	PIPC+GM	IPM/CS+CAZ+GM
Outcome	Recovered	Recovered	Recovered

Abbreviations: APL, acute promyelocytic leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CR, complete remission; DIC, disseminated intravascular coagulation; FOM, fosfomycin; GM, gentamicin; CMZ, cefmetazole; PIPC, piperacillin; CAZ, ceftazidime; and IPM/CS, imipenem/cilastatin.

*¹Rise of at least 100/ μ l in granulocyte count at 14 days after onset.

Recombinant human granulocyte colony-stimulating factor was administered subcutaneously at a daily dose of 75 μ g/body prior to the onset of bacteremia in Case J.O.

*²Antibiotics before positive blood cultures were obtained.

intensive induction chemotherapy with resultant profound granulocytopenia and complained of oral pain so severe as to make opening the mouth difficult. Exacerbation of previously asymptomatic periodontitis occurred in two patients, while severe oral mucositis associated with chemotherapy was observed in the other one. Antibiotic therapy was started on an empirical basis. Blood cultures grew *C. sputigena* after four to five days of incubation. Two isolates showed β -lactamase production, and the other, a non- β -lactamase-producing one, was obtained from a patient (M.M.) who did not receive a β -lactam antibiotic because of a history of allergy to cefoperazone. Prior antibiotic treatment thus proved ineffective. However, all patients showed clinical improvement after the institution of appropriate therapy in association with bone marrow recovery. The clinical picture seemed to be similar to that of bacteremia due to any other gram-negative organism. Neither shock nor coagulopathy supervened.

Bacteriological Studies Materials and Methods

Blood Cultures

The blood sample (5–10 ml) was equally inoculated into two kinds of culture bottles: Liquoid Blood Culture Bottles TSB and Thio (Roche) (tryptic soy broth and thioglycolate medium, respectively), which were incubated at 37°C for at least seven days.

Tests for Biochemical Characterization of *Capnocytophaga* Isolates

The three blood culture isolates were identified, at the generic level, as *Capnocytophaga* species by

conventional methods^{1,9,10)} as well as by the RapID ANA II System® (Innovative Diagnostic Systems, Atlanta, Georgia), which was used according to the manufacturer's directions. The agar media used were trypto-soy agar (Eiken) supplemented with 5% sheep blood (TS-blood agar and chocolate agar), MacConkey agar (Eiken) and GAM agar (Nissui). Incubation temperature was 37°C, except that growth was tested at 42°C¹⁰⁾. GasPak Anaerobic Systems® (BBL) were used to create anaerobic and capneic (5% CO₂) conditions. The latter atmosphere was achieved by the use of jars without catalysts.

For species delineation of the *Capnocytophaga* isolates, additional biochemical tests were carried out. Fermentation tests for carbohydrates (glucose, lactose, mannitol, sucrose, maltose, xylose, arabinose, cellobiose, glycerol, raffinose, rhamnose, salicin, sorbitol and trehalose) were performed using the API-20A® system for the identification of anaerobes (API Systems S.A., France) according to the manufacturer's directions. Acid production from and/or hydrolysis of other carbohydrates (fructose, galactose, dextran and starch) were studied according to the description in the Anaerobe Laboratory Manual of Virginia Polytechnic Institute¹¹⁾, using GAM semisolid medium (Nissui) as a basal medium. The isolates were tested for gelatin and starch hydrolysis and nitrate reduction by the methods described in Cowan and Steel's Manual¹²⁾. Major acid end products of metabolism in GAM semisolid glucose medium (Nissui) were analyzed by gas-liquid chromatography¹¹⁾.

Deoxyribonucleic Acid (DNA) Base Composition

Mol-percent guanine and cytosine (% G + C) was determined by high-performance liquid chromatography¹³⁾.

DNA Homology

Capnocytophaga isolates were speciated on the basis of relative homology values as compared with the type strain of each species. The microplate DNA-DNA hybridization method described by Ezaki et al.¹⁴⁾ was used to demonstrate DNA homology. The hybridization experiment was carried out at 50°C for two hours. Reported results are the average of four such experiments. When the type strain and clinical isolates showed more than 70% DNA homology, they were considered to belong to the same species. The type strains used were *Capnocytophaga ochracea* ATCC 27872, *C. sputigena* ATCC 33612, *Capnocytophaga gingivalis* ATCC 33624, and *Capnocytophaga cynodegmi* ATCC 49044.

Antimicrobial Susceptibility

Susceptibility testing on TS-blood agar was performed in a capneic atmosphere by the triple disk method using Tridisks (Eiken). After 72 hours of incubation at 37°C, the results were expressed in a four-grade rating of sensitivity from – to ‡. In addition, susceptibility to vancomycin was determined by the Kirby-Bauer method¹⁵⁾ using Sensi-Disc (BBL). The results were classified into three categories of susceptibility: resistant (R), intermediate (I) and sensitive (S).

Production of β -lactamase was determined by the chromogenic cephalosporin (nitrocefin) method using Cefinase (BBL).

Results

Cultural Findings of *Capnocytophaga* Isolates

Growth in the blood culture bottles became visible after four to five days of incubation in all three cases.

Morphological and physiological characteristics of the three isolates are shown in Table 2. On TS-blood agar, they grew equally well in a capneic and an anaerobic atmosphere. In contrast, they did not grow on GAM agar in a capneic atmosphere, although active anaerobic growth was seen on the same agar. However, capneic growth on GAM agar was obtained only after a passage on TS-blood agar under the same conditions. Gliding colonies, whether on TS-blood agar or on GAM agar, were easily observed by oblique

Table 2 Characteristics of three blood culture isolates*¹

	Reaction		Reaction
Gram reaction	—	Acid from	
Fusiform or		Glucose	+
rod-shaped cells	+	Glycerol	—
Colony pigmentation		Lactose	+
(yellow orange)	+	Maltose	+
Bitter almond odor	+	Mannose	+
Gliding motility	+	Raffinose	+
Growth at 37°C on		Sorbitol	—
Blood agar		Sucrose	+
In air	—* ²	Trehalose	—
Air + 5 % CO ₂	+	Xylose	—
GasPak atmosphere	+	Arabinose	+ (1), — (2)
Chocolate agar	+	Mannitol	+ (1), — (2)
GAM agar	+* ³	Melezitose	+ (1), — (2)
MacConkey agar	—	Rhamnose	+ (1), — (2)
Growth at 42°C	+	Cellobiose	+ (2), — (1)
Oxidase	—	Salicin	+ (2), — (1)
Catalase	—	Fructose	—
Urease	—	Galactose	—
Indole	—	Dextran	—
Nitrate reduction	+	Starch	+
Hydrolysis of		Acid end products	AS(iv)**
Esculin	+	Genus suggested by	
Starch	— (2), + (1)	RapID ANA II System :	
Gelatin	—	Capnocytophaga species	

*¹Symbols: —, all isolates were negative; +, all isolates were positive. When different reactions occurred in different isolates, figures in parentheses indicate no. of isolates which showed their respective symbols.

*²Aerobic subculture yielded some small, non-spreading colonies only after 72 to 96 hours of incubation.

*³Capneic growth was obtained only after at least one passage on TS-blood agar in air with 5 % CO₂, although anaerobic growth was always active.

*⁴Fatty acids were detected by gas-liquid chromatography (GLC). Capital and small letters indicate major and minor peaks, respectively.

A : acetic acid; S : succinic acid; and iv : isovaleric acid. GLC analysis of cultures from one patient (Y.K.) revealed trace amounts of isobutyric acid as well.

illumination. Aerobic culture on TS-blood agar yielded a growth of small, yellow-orange, non-gliding colonies after 72 to 96 hours of incubation. Gram staining showed a morphological change from rod-shaped cells in younger cultures of 24 to 48 hours' incubation to fusiform cells in older cultures of more than 48 to 96 hours' incubation, irrespective of the atmospheric condition, whether capneic or anaerobic. Curved forms were also present in small numbers.

Species Differentiation

Because of the phenotypical similarity of *Capnocytophaga* species, it proved difficult to speciate the three isolates by conventional techniques used in most hospital microbiology laboratories (Table 2.) In contrast, DNA homology studies identified all isolates as *C. sputigena* (Table 3).

Antimicrobial Susceptibility

The susceptibility of the three isolates to 21 antimicrobial agents is listed in Table 4. Among the β -lactam antibiotics tested, clavulanic acid/amoxicillin, cefotaxime and imipenem proved active against all

Table 3 Genomic speciation of *Capnocytophaga* isolates

Isolate	M.M.	Y.K.	J.O.
DNA base composition ^{*1}			
%G+C	38.7	38.8	Not done
DNA homology ^{*2}			
Percent hybridization with DNA from			
<i>C. ochracea</i> ATCC 27872	60	25	53
<i>C. sputigena</i> ATCC 33612	100	100	85
<i>C. gingivalis</i> ATCC 33624	2	3	36
<i>C. cynodegmi</i> ATCC 49044			15
<i>Escherichia coli</i> K-12	0	0	
Salmon-sperm DNA			0
Genomic speciation	<i>C. sputigena</i>	<i>C. sputigena</i>	<i>C. sputigena</i>

^{*1}Mol-percent guanine and cytosine (% G+C) was determined by high-performance liquid chromatography¹³⁾.

^{*2}Relative homology values were determined by the microplate hybridization method¹⁴⁾.

Table 4 *In vitro* susceptibility of *C. sputigena* isolates to antimicrobial agents

Isolate	M.M.	Y.K.	J.O.
ABPC ^{*1}	++	++	—
CVA/AMPC	++	++	++
CBPC	++	++	+
PIPC	++	++	—
CEZ	++	+	—
CMZ	++	+	++
CPZ	++	+	—
SBT/CPZ	++	++	++
CTX	++	++	++
CAZ	++	++	—
LMOX	++	++	++
IPM	++	++	++
AZT	++	++	++
FOM	—	—	—
GM	+	++	++
AMK	+	++	++
MINO	++	++	++

CLDM	++	++	++
CL	—	—	—
NFLX	++	++	++
VCM ^{*2}	S	S	S

Nitrocefin test	—	+ ^{w*3}	+
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Abbreviations: ABPC, ampicillin; CVA/AMPC, clavulanic acid/amoxicillin; CBPC, carbenicillin; PIPC, piperacillin; CEZ, cefazoline; CMZ, cefmetazole; CPZ, cefoperazone; SBT/CPZ, sulbactam/cefoperazone; CTX, cefotaxime; CAZ, ceftazidime; LMOX, latamoxef; IPM, imipenem; AZT, aztreonam; FOM, fosfomycin; GM, gentamicin; AMK, amikacin; MINO, minocycline; CLDM, clindamycin; CL, colistin; NFLX, norfloxacin; and VCM, vancomycin.

^{*1}Susceptibility to antimicrobial agents other than vancomycin was determined by the triple disk method using Tridisks (Eiken).

^{*2}Susceptibility to vancomycin was tested by the Kirby-Bauer method¹⁵⁾ using Sensi-Disc (BBL).

^{*3}Weakly positive reaction

isolates, two of which gave a positive nitrocefin test. The activity of other β -lactam agents varied depending largely upon the β -lactamase-producing ability of the isolates tested. One of the β -lactamase-producing isolates proved resistant to both piperacillin and ceftazidime, either of which is currently used in combination with an aminoglycoside for the treatment of febrile patients with granulocytopenia. Moreover, all isolates were susceptible to clindamycin, minocycline, vancomycin and norfloxacin, while they were resistant to fosfomycin and colistin, and to a lesser extent, to gentamicin and amikacin. Incidentally, the aminoglycosides became less active in anaerobic incubation.

Discussion

Isolation of *Capnocytophaga* species from previously sterile sites such as blood is usually considered to

be a potential clue that the source is oral^{4,5}), since the organisms are typical components of the oral microflora¹⁻³). Oropharyngeal mucosal breakdown as a consequence of intensive chemotherapy promotes access of the resident flora to deeper tissues. On the other hand, exacerbation of previously asymptomatic periodontal disease has recently been noted to occur with increasing frequency in patients with acute leukemia receiving induction chemotherapy, in contrast to the marked decrease in the number of lower alimentary tract infections⁶). In such clinical settings, the incidence of capnophilic and anaerobic bacteremia originating from the oropharynx is inferred to have increased in granulocytopenic patients, especially those with hematologic malignancy^{1,4,8}).

The empiric antibiotic regimen currently recommended by the European Organization for Research on Treatment of Cancer consists of ceftazidime plus amikacin¹⁶), both of which have little or no activity against most of the capnophilic and anaerobic isolates from the blood of granulocytopenic patients^{4,17}). An increasing frequency of β -lactamase-producing *Capnocytophaga* isolates has been noted in recent years^{4,17}), as already reported for *Bacteroides* species^{7,8}). Many of the antibiotics effective against the latter are active against the former⁴). Therefore, the empiric use of antibiotics with anti-anaerobic activity, such as imipenem and clindamycin, may be justified when granulocytopenic patients with severe chemotherapy-induced mucositis or acute exacerbation of periodontal disease develop a persistent fever of unknown origin^{4,7}).

Because of the phenotypical resemblance of *Capnocytophaga* species^{9,10}), it seems impossible in practice to identify clinical isolates on a species level by the conventional biochemical tests used in most hospital microbiology laboratories^{10,18,19}). This is true of the distinction between *C. sputigena* and *C. ochracea*, although tests for starch and gelatin hydrolysis have been reported to be helpful in distinguishing these two species¹). As stated by Williams et al.¹⁸) and Speck et al.¹⁹), therefore, DNA homology studies are considered indispensable for species differentiation of *Capnocytophaga* isolates.

References

- 1) Holt, S.C. & Kinder, S.A.: *Capnocytophaga*. In Bergey's Manual of Systematic Bacteriology. (Staley, J.T. ed.) p. 2050—2058, Williams & Wilkins, Baltimore, 1989.
- 2) Newman, M.G. & Sims, T.N.: The predominant cultivable microbiota of the periodontal abscess. J. Periodontol. 50: 350—354, 1979.
- 3) Holdeman, L.V., Moore, W.E.C., Cato, E.P., Burmeister, J.A., Palcanis, K.G. & Ranney, R.R.: Distribution of *Capnocytophaga* in periodontal microfloras. J. Periodont. Res. 20: 475—483, 1985.
- 4) Baquero, F., Fernández, J., Dronda, F., Erice, A., Pérez de Oteiza, J., Reguera, J.A., & Reig, M.: Capnophilic and anaerobic bacteremia in neutropenic patients: an oral source. Rev. Infect. Dis. 12 (Suppl. 2): S157—S160, 1990.
- 5) Parenti, D.M. & Snyderman, D.R.: *Capnocytophaga* species: infections in nonimmunocompromised and immunocompromised hosts. J. Infect. Dis. 151: 140—147, 1985.
- 6) Overholser, C.D., Peterson, D.E., Williams, L.T. & Schimpff, S.C.: Periodontal infection in patients with acute nonlymphocytic leukemia. Prevalence of acute exacerbations. Arch. Intern. Med. 142: 551—554, 1982.
- 7) Fainstein, V., Elting, L.S. & Bodey, G.P.: Bacteremia caused by non-sporulating anaerobes in cancer patients. Medicine 68: 151—162, 1989.
- 8) Funada, H., Machi, T. & Matsuda, T.: Anaerobic bacteremia in patients with hematologic malignancy. Kenkiseikin-Kansensho-Kenkyu (J. Jpn. Assoc. Anaerobic Infect. Res.) 17: 12—23, 1987.
- 9) Socransky, S.S., Holt, S.C., Leadbetter, E.R., Tanner, A.C.R., Savitt, E. & Hammond, B.F.: *Capnocytophaga*: new genus of gram-negative gliding bacteria. III. Physiological characterization. Arch. Microbiol. 122: 29—33, 1979.
- 10) Kristiansen, J.E., Bremmelgaard, A., Busk, H.E., Heltberg, O., Frederiksen, W. & Justesen, T.: Rapid identification of *Capnocytophaga* isolated from septicemic patients. Eur. J. Clin. Microbiol. 3: 236—240, 1984.
- 11) Holdeman, L.V., Cato, E.P. & Moore, W.E.C. (ed.): Anaerobe Laboratory Manual, 4th ed. Virginia Polytechnic Institute Anaerobe Laboratory, Blacksburg, Va., 1977.
- 12) Cowan, S.T.: Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd ed., Cambridge University Press, London, 1974.

- 13) Kaneko, T., Katoh, K., Fujimoto, M., Kumagai, M., Tamaoka, J. & Katayama-Fujimura, Y.: Determination of the nucleotide composition of a deoxyribonucleic acid by high-performance liquid chromatography of its enzymatic hydrolysate: a review. *J. Microbiol. Methods* 4: 229—240, 1986.
- 14) Ezaki, T., Hashimoto, Y. & Yabuuchi, E.: Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* 39: 224—229, 1989.
- 15) Bauer, A.W., Kirby, W.M.M., Sherris, J.C. & Turck, M.: Antibiotic susceptibility testing by a standardized disk method. *Am. J. Clin. Pathol.* 45: 493—496, 1966.
- 16) The EORTC International Antimicrobial Therapy Cooperative Group: Ceftazidime combined with a short or long course of amikacin for empirical therapy of gram-negative bacteremia in cancer patients with granulocytopenia. *N. Engl. J. Med.* 317: 1692—1698, 1987.
- 17) Arlet, G., Sanson-Le Pors, M-J, Casin, I.M., Ortenberg, M. & Perol, Y.: *In vitro* susceptibility of 96 *Capnocytophaga* strains, including a β -lactamase producer, to new β -lactam antibiotics and six quinolones. *Antimicrob. Agents Chemother.* 31: 1283—1284, 1987.
- 18) Williams, B.L., Hollis, D. & Holdeman, L.V.: Synonymy of strains of Center for Disease Control group DF-1 with species of *Capnocytophaga*. *J. Clin. Microbiol.* 10: 550—556, 1979.
- 19) Speck, H., Kroppenstedt, R.M. & Mannheim, W.: Genomic relationships and species differentiation in the genus *Capnocytophaga*. *Zbl. Bakt. Hyg. A* 266: 390—402, 1987.

急性白血病の治療中に合併した *Capnocytophaga sputigena* 菌血症

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要 旨

過去3年間(1989～1991年)に、*Capnocytophaga* 菌血症が3例の急性白血病の治療中に発症した。侵入門戸は口腔内病変(歯周炎や重症の粘膜炎)と考えられた。血液から分離された3菌株はデオキシリボ核酸(DNA)の相同性から *Capnocytophaga sputigena* と同定された。しかし、*Capnocytophaga* の生物学的性状の類似性から、分離菌株の菌種を通常の臨床細菌学的検査法で同定することは困難であった。血液分離菌は嫌気性菌に

通常抗菌力をもつ抗生物質の大部分に感性を示した。 β -ラクタマーゼ産生が2菌株に認められ、このうちの1菌株は顆粒球減少患者の発熱時に経験的に使用されることの多いピペラシリン(piperacillin)とセフトジジム(ceftazidime)の両薬に耐性であった。それゆえ、抗腫瘍薬治療による重症の口腔粘膜炎や歯周炎の急性増悪をみる顆粒球減少患者の発熱時にはイミペネム(imipenem)やクリンダマイシン(clindamycin)の使用が考慮されるべきである。