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研究報告の概要

⊙Propionibacterium acnesは、濃厚血小板製剤中で増殖しない

背景および目的:Propionibacterium acnes(P. acnes)は、嫌気性培養による検出方法を用いた場合、血小板濃縮製剤(PC)でもっとも頻度の高い汚染菌のひとつと見なされている。しかし、プロピオン酸菌属は、すでに血液製剤が輸血された後で検出される場合が多い。また、P. acnes汚染PCを輸血された患者の転帰についての試験は現在もあまり行われていないことから、P. acnesと輸血の関連性の解明が望まれている。本試験では、輸血後に無菌試験で細菌が検出されたPCの受血者の臨床効果のモニタリングを行った。さらに、血小板細菌スクリーニングにおけるプロピオン酸菌属の重要性を明らかにするために、PCに接種したプロピオン酸菌属の細菌増殖を評価した。

材料および方法:ルックバック調査において、汚染が推定されるPCの保存から輸血までの経路を追跡した。In vitro試験ではPCにプロピオン酸菌属の臨床分離菌1～100 CFU/mLを接種した(n=10)。好気的に22℃で10日間保管している間にサンプルを摂取し、平板培養および自動BacT/Alert培養システムにより、細菌の有無を評価した。

結果:P. acnesは、PC保存条件下では、細菌の生育は緩慢であるか、または生育を認めなかった。汚染の可能性のあるPCを輸血した後の副作用は認めなかった。

結論:プロピオン酸菌属はPC保存条件下で増殖しないために、検出されないか、血液製剤がすでに輸血された後に検出されると考えられた。

報告企業の意見	今後の対応
Propionibacterium acnesをはじめとするプロピオン酸菌属は、濃厚血小板製剤の保存条件下では増殖せず、汚染の可能性のある製剤を輸血した後の副作用は認めなかったとの報告である。	日本赤十字社では、輸血による細菌感染予防対策として平成18年10月より血小板製剤について、また、平成19年3月より全血採血由来製剤について、初流血除去を導入した。また、全ての輸血用血液製剤について、平成19年1月より保存前白血球除去を実施している。さらに、輸血情報リーフレット等により細菌感染やウイルス感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。今後も細菌やウイルスの検出や不活化する方策について情報の収集に努める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

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Propionibacterium acnes lacks the capability to proliferate in platelet concentrates

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Vox Sanguinis

Background and Objectives *Propionibacterium acnes* is considered to be one of the most frequent contaminants of platelet concentrates (PCs) when anaerobic culture-based detection methods are used. But *Propionibacteria* are often detected too late when blood products have already been transfused. Therefore, its transfusion relevance is still demanding clarification because studies of the outcome of patients transfused with *P. acnes*-contaminated PCs are still uncommon. In this study, we monitored clinical effects in patients after transfusion of PCs, which were detected too late in sterility testing. Furthermore, we assessed the bacterial proliferation of *Propionibacterium* species seeded into PCs to clarify their significance for platelet bacteria screening.

Materials and Methods In the look-back process, we followed the route of the putative contaminated PC units from storage to transfusion. In the *in vitro* study, PCs were inoculated with 1–100 colony-forming unit (CFU)/ml of clinical isolates of *Propionibacteria* ($n = 10$). Sampling was performed during 10-day aerobic storage at 22 °C. The presence of bacteria was assessed by plating culture and automated BacT/Alert culture system.

Results *Propionibacterium acnes* shows slow or no growth under PC storage conditions. Clinical signs of adverse events after transfusion of potentially contaminated PC units were not reported.

Conclusion *Propionibacteria* do not proliferate under PC storage conditions and therefore may be missed or detected too late when blood products have already been transfused.

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Key words: automated culture, bacterial detection, platelet contamination, *Propionibacterium acnes*, sterility testing.

Introduction

Bacterial contamination of platelet concentrates (PCs) is an ongoing problem associated with significant transfusion-related morbidity and mortality. Currently, PC transfusion-transmitted sepsis is recognized as the most frequent infectious complication in transfusion therapy, surpassing by up to two orders of magnitude the incidence of transfusion-associated viral transmission [1,2]. Most reports estimate that as many as 1 in 2000 to 3000 PCs, both apheresis-derived and buffy-coat-derived PCs are contaminated with bacteria [1,3]. Due to their storage at room temperature for up to 5 days, PCs are the most frequently affected blood product [1,4]. These conditions permit growth of bacteria with the potential for transmission to patients receiving platelet preparations [5]. Next to coagulase-negative *Staphylococci*, *Propionibacterium acnes* is implicated in most cases of bacterial contamination of PCs and is detected fairly frequently when anaerobic bottles are used [1,3,5,6]. Schmidt *et al.* [7] reported 20 of 37 initial

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Abbreviations: DSM, Deutsche Stammsammlung für Mikroorganismen; IP, Propionibacterium isolate; OWL, Ostwestfalen-Lippe; PVX, PolyVitex; PCs, platelet concentrates; PBS, phosphate-buffered saline.

positive anaerobic cultures of which three were confirmed positive on reculture for *P. acnes* while Schrezenmeier *et al.* [8] reported 45 of 98 initial anaerobic positive samples with 20 confirmed positive on reculture for *P. acnes*. It accounts for approximately half of the total skin flora, with an estimated density of 10^2–10^6 organisms per cm^2 [9]. Accordingly, the bacterial entry from venepuncture during a conventional blood donation is expected to be 0·03 colony-forming unit (CFU)/ml [10]. Therefore, the donor phlebotomy site represents the major source of bacterial contamination of PCs [8]. In the UK, Serious Hazards of Transfusion (SHOT) reports that potentially 80% of bacterial transmissions, in which the source was defined, were derived from the donor's arm [11,12].

Propionibacterium acnes is a Gram-positive, slow-growing, non-sporeforming anaerobic bacterium that is commonly present as part of the normal skin flora and colonizes within the sebaceous glands, which are the likely sites of platelet contamination with a density of 10^2–10^3 organisms per cm^2. Even a careful disinfection of the donor phlebotomy site using a single-swab method with 70% isopropyl alcohol may result in incomplete disinfection of such organisms [13]. de Korte and colleagues [14] reported that surface disinfection will therefore be less adequate to remove diphtheroids like *P. acnes*, whereas diversion of the first 10 ml of a whole-blood donation will reduce all kind of skin flora. Limited reports have pointed out that *P. acnes* can be causative for a variety of infections, including endophthalmitis, neurosurgical wound infections, pulmonary infections and endocarditis. But, primarily it is considered as a contaminant of cultures obtained percutaneously, including blood cultures [15].

Since screening for bacterial contamination was recommended by the American Association of Blood Banks, several technologies including culture and rapid methods for bacterial detection have been developed [10,11,16]. Most facilities have adopted the semiautomated BacT/Alert 3D culture system (bioMérieux, Nürtingen, Germany), which is cleared for the quality control of PCs by the Food and Drug Administration (FDA), as the instrument to detect platelet contamination [17]. But despite the success of prevention of transfusion-transmitted infections, continued reports raise the possibility that this system has disadvantages and an appreciable failure rate [17–19]. On the one hand, slow-growing organisms may be detected after the product has already been transfused; on the other hand, two-bottle blood-culture systems allow for optimized growth of both aerobic and anaerobic organisms yet also enable detection of bacterial strains that are unable to proliferate in human PCs. Nevertheless, improvements from increasing the sensitivity and speed of this detection method are under development. Brecher and Hay [20] argue for the routine implementation of an anaerobic bottle together with an aerobic bottle for the detection of platelet bacteria contamination because of the great diversity of bacterial preferences for growth in either aerobic or anaerobic bottles. The addition of the anaerobic bottle slightly improves the time to first detection of some facultative anaerobes [20] and allows detection of obligate anaerobes, which have infrequently been implicated in transfusion-mediated bacterial sepsis [21]. Furthermore, doubling the platelet sample volume improves the detection of slow-growing organisms by approximately 25% [22].

In general, studies about bacterial contamination of PCs emphasize the incidence of *Propionibacteria* in platelet bacteria screening using automated culture but to date the significance of this organism in platelet bacteria screening is still not clear and badly needs clarification. Therefore, we monitored the clinical patients' outcome after transfusion of an initially culture-positive PC to clarify the clinical relevance of *P. acnes*. Moreover, we determined the bacterial growth kinetics of *Propionibacterium* species in PCs during storage. Subsequently, the significance of culture-positive detection at the end of PCs storage in platelet bacteria screening shall be discussed.

Materials and methods

Blood collection

Apheresis-derived single-donor platelets were obtained from the transfusion service UniBlutspendedienst Ostwestfalen-Lippe, Bad Oeynhausen, Germany, after standard processing with the Haemonetics MCS+ (Haemonetics GmbH, München, Germany) from healthy blood donors and stored at 20 to 24 °C with agitation. Predonation sampling was performed after donor arm disinfection using a single-swab method with 70% isopropyl alcohol.

Source of *Propionibacterium* isolates – routine sterility testing of PCs

This study was conducted with isolates of *Propionibacterium* (IP) species ($n = 6$; isolates IP540, IP240, IP016, IP551, IP095 and IP816), which were isolated from contaminated PCs during routine sterility testing of PCs at our transfusion service. All six cases of *P. acnes* were detected only in the anaerobic bottle in the automated culturing system. For routine screening of PCs, 15 ml of sample is taken under aseptic conditions after standard processing of PCs and storage of up to 24 h at 22 °C with agitation, and is used for microbial and molecular genetic sterility testing as described by Störmer *et al.* [23]. For this purpose, nucleic acids are extracted using magnetic separation technology (Chemagen, Baesweiler, Germany) and analysed by a one-step reverse transcriptase-polymerase chain reaction (RT-PCR) method using a primer and probe system for amplifying a 122-bp fragment of bacterial 23S ribosomal RNA. As an internal extraction and amplification control, human β_2-microglobulin (B2-MG) mRNA was coextracted and coamplified with each reaction to avoid

false-negative results due to PCR inhibition. The BacT/Alert (bioMérieux) automated culturing system served as reference method where 5 ml of PCs were inoculated into both the aerobic (BacT/Alert BPA; bioMérieux) and standard anaerobic culture bottle (BacT/Alert BPN) and were incubated for up to 7 days. Initial reactive [7] anaerobic culture bottles (BacT/Alert BPN; bioMérieux) were subcultured and the identification of bacterial isolates was performed by 16S rRNA analysis and biochemical tests.

In addition, *P. acnes* (IP3912), *Propionibacterium avidum* (IP4851) and *Propionibacterium granulosum* (IP5152) isolated from other clinical samples and reference strain *P. acnes* DSM (Deutsche Stammsammlung für Mikroorganismen) 1897, which was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Heidelberg, Germany), were included in this study. The 10 *Propionibacterium* strains were cultured in Trypticase Soy Broth (TS; bioMérieux) at 37 °C under anaerobic conditions for 48 h. Serial 10-fold dilutions of grown cultures were made in phosphate-buffered saline (PBS) and plated on PolyVitex (PVX) blood agar plates (PVX; bioMérieux) to determine the bacterial titre (CFU/ml). Aliquots, taken from appropriate dilutions, were used for inoculation of the PCs.

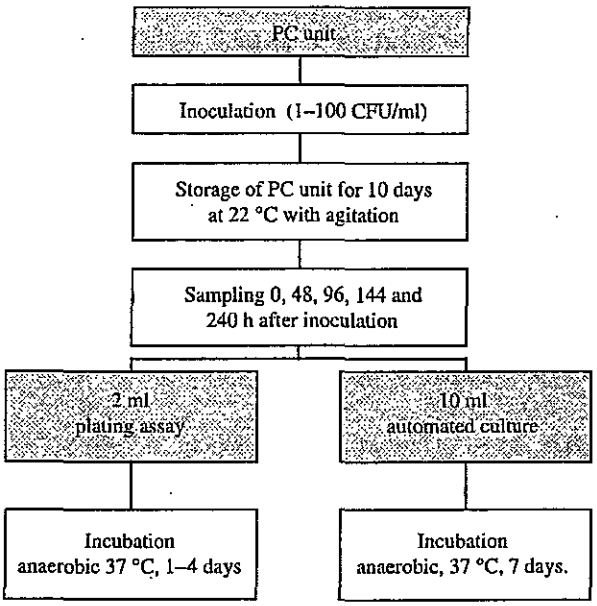

Fig. 1 Flow diagram representing the inoculation and sampling strategy. Inoculation of one single apheresis-derived platelet concentrate (PC) for one *Propionibacterium* species, and sampling for BacT/Alert are displayed.

Propionibacteria identification

Isolates of *Propionibacteria* were biochemically identified by using the API 20A multitest identification system (bioMérieux) in accordance with the manufacturer's instructions. For molecular genetic identification, PCR was performed using universal primers described by Ley *et al.* [24], which targets a conserved region of 16S ribosomal DNA. DNA sequencing and analysis was performed as described previously [25]. Sequence data have been submitted to GenBank and assigned accession numbers EF670439 to EF670442, EF670445, EF670450, EF680378 to EF680380, and EF680382.

Look-back process

In our PC-screening programme, we found six PCs tested positive for *P. acnes* [23]. In the look-back process, we followed the route of these putative contaminated PC units from storage to transfusion and monitored the clinical characteristics of the recipients. The donor directed look-back process summarized the detection time in the BacT/Alert system in relation to the time of transfusion of PCs. We reviewed the medical records of the six patients that received PCs tested positive for *P. acnes* in the BacT/Alert system. Medical records and laboratory information system searches were abstracted for primary diagnoses, kind of surgery, age at transfusion, microbiological findings, antibiosis at transfusion and markers of inflammatory events [C-reactive protein (CRP), leucocytes].

Inoculation and bacterial monitoring

To determine the growth kinetics of the isolated *P. acnes* strains during PC storage, we spiked PC units and monitored the presence of *P. acnes* during storage at 22 °C. All PCs used were sampled before bacterial inoculation to assure baseline sterility of the original apheresis bags. For this reason, 5 ml were inoculated into both the aerobic (BacT/Alert BPA; bioMérieux) and standard anaerobic culture bottles (BacT/Alert BPN) and incubated for up to 7 days.

For each bacterial strain, one PC was spiked with 1–100 CFU/ml of *Propionibacterium* species as shown in Fig. 1. To ensure the presence of *Propionibacteria* in the inoculated PC unit, a sample was taken immediately after inoculation (0 h) and analysed with the BacT/Alert 3D continuous monitoring system (bioMérieux).

To monitor the presence and proliferation of *Propionibacteria* in PCs by proliferation testing on blood agar plates and automated culture, sampling was performed during the 10-day storage at 22 °C with agitation at 48, 96, 144 and 240 h after inoculation. For this purpose, 5 ml aliquots of each PC unit were transferred in duplicate to the standard anaerobic culture bottle (BacT/Alert BPN). Incubation was performed using the BacT/Alert 3D continuous monitoring system at 37 °C until a reactive signal was detected, or for up to 7 days, if the signal remained negative. Samples that did not react after 7-day storage were considered sterile. Initially, reactive culture bottles were subcultured for confirmation and identification of *Propionibacteria*. Moreover, for visual inspection

and determination of the bacterial titre, 100 µl aliquots of serial dilutions of PC samples were plated in triplicate onto PVX blood agar and incubated at 37 °C for 48–168 h. To detect a bacterial level below 10 CFU/ml, 1 ml of sample was plated onto PVX blood agar, as well. After incubation, the number of colonies was counted and the concentration of *Propionibacteria* per ml of sample was calculated. Furthermore, to exclude donor-specific factors, like the presence of neutralizing antibodies, two further PC units from different donors were spiked with each *Propionibacterium* strain and bacterial proliferation was monitored by plate culture. All procedures were performed under sterile laminar air flow conditions.

Results

Study design

A total of 1533 apheresis-derived PC units were screened for bacterial contamination during a 20-month study period in our facility by automated culture and real-time RT-PCR as described previously [23]. In accordance with the definitions used by Schmidt *et al.* [7], we considered samples without a positive reaction in either test as negative. Samples with a reactive signal but no microbiological confirmation of the bacterial strain were labelled as initially reactive. Hence, a sample with both a reactive signal and microbiological confirmation was regarded as initially positive. Correspondingly, six anaerobic culture bottles were identified by the automated culture system as being initially positive (0·39%). An aliquot was removed from the initially positive culture bottle for Gram-staining and subculture to agar media. The six isolates were identified as *P. acnes* by biochemical and molecular genetic identification in all six cases. All strains were detected by the automated culture system between 5 and 6 days (5·19 ± 0·79) after sampling, or 6 and 7 days (6·19 ± 0·79) after donation, respectively. At that time, the platelet product had already been transfused and no sample or predonation bag was available for confirmation of the positive result, but no adverse reactions were noted after transfusion.

Look-back process

Because of the late detection of the automated culture system in our platelet bacteria screening study, all PCs had been transfused. Putative contaminated PCs were transfused within the first day ($n = 3$), second day ($n = 2$) and third day ($n = 1$) of storage whereas the BacT/Alert culture system detected these PC units between 5 and 6 days after donation. To exclude bacteraemia of the PC donors, the following PC donations were especially monitored for bacterial contamination using microbial and molecular genetic sterility testing, but without positive confirmation. In the look-back process, we reviewed the medical records of six patients that received PCs tested positive for *P. acnes* in the BacT/Alert system as shown in Table 1. All transfusion reports were returned to the blood bank and transfusion was documented without complications. Back-tracked PCs were transfused perioperatively or postoperatively to massively bleeding patients who underwent heart surgery. Because of bacterial infectious diseases prior to transfusion, most patients ($n = 5$) were under antibiotic therapy with drugs that should be effective against *P. acnes* as well. One patient was under immunosuppressant therapy due to heart transplantation. The progression of proinflammatory markers [procalcitonin (data not shown), CRP (reference range ≤ 5 mg/l) or leucocyte count] has to be regarded as crucial because of prior bacterial infectious diseases. Furthermore, the increase of these markers may be the result of a postoperative acute phase reaction. Blood cultures taken after transfusion of the PC unit were sterile.

Growth characteristics of *Propionibacteria* in platelet concentrates

In order to assess the bacterial proliferation of *Propionibacterium* species in PCs under storage conditions, the presence of bacteria was monitored by plate culture and enrichment culture as shown in Fig. 1. The results of the investigation are shown in Fig. 2. Sampling time, bacterial load (growth-curve of *Propionibacteria*) and detection time of the BacT/Alert culture system are presented for each *Propionibacterium* strain. Approximately 24 h after donation, PCs from different donors were spiked with one of the 10 *Propionibacterium* strains and bacteria contents were monitored by colony-forming assay and automated culture during a 10-day storage. The mean initial bacterial inoculum densities at the beginning of storage (day 0) for the PCs were determined by colony-forming assay and varied between 2 and 80 CFU/ml. Following inoculation, a slight increase to approximately 150 CFU/ml, a subsequent decrease or no change of the bacterial load were observed during storage at 22 °C depending on the *Propionibacterium* strain.

Propionibacterium isolates IP540, IP551, IP816 and IP095 showed a slightly increased bacterial load in the first 48 h of PC storage that decreased down to 10 CFU/ml in the following days. The bacterial load of the isolates IP016 and IP240 were already slightly reduced after 48 h and remained unchanged as well as for isolates IP3912, DSM1897, IP4851 and IP5152. Therefore, all *Propionibacteria* strains showed no proliferation in the PC within the 10 days. The influence of donor-specific factors was excluded, because all *Propionibacterium* strains showed similar growth kinetics in PC units from different donors (data not shown).

Automated culture monitoring of bacterial growth

As shown in Fig. 2, all day 0 inoculated samples cultured in the anaerobic bottles were signaled positive by the automated

Table 1 Outcome of recipients of putative contaminated platelet concentrate (PC) units

Donor							Recipient							
P. acnes isolate	Donor sex (age/years)	Time of donation	Time of TF[a]	Aerobic culture detection	Anaerobic culture detection[b]	Bacterial strain	Recipient sex (age/years)	Disease and surgical intervention	Microbiological diagnostic findings after TF	Antibiosis	CRP[c] pre-TF (mg/dl)	CRP post-TF (mg/dl)	Leucocytes pre-TF (10^9/l)	Leucocytes post-TF (10^9/l)
IP016	Female (32)	10 May 2006	12 May 2006	Negative[d]	Positive 107 h (5 days) (16 May 2006)	*P. acnes*	Male (77)	Aortic and mitral valve replacement, aortic plastic valvular prosthesis, ancurysma aorta acendes	Urinary tract infection with *P. aeruginosa* and *E. faecium*	No	0·38 (11 May 2006)	6·68 (13 May 2006)	6·4 (11 May 2006)	13·1 (13 May 2006)
IP540	Female (41)	23 October 2006	24 October 06	Negative	Positive 113 h (5 days) (29 October 2006)	*P. acnes*	Male (62)	Coronary heart disease, heart transplantation	Blood culture negative (2 November 2006)	Yes (vancomycin, imipenem)	0·74 (23 October 2006)	0·53 (24 October 2006)	7·7 (23 October 2006)	13·0 (24 October 2006)
IP551	Male (28)	26 October 2006	28 October 2006	Negative	Positive 159 h (6 days) (2 November 2006)	*P. acnes*	Female (87)	Aortic valve stenosis, aortic plastic valvular prosthesis aortic valve replacement aortocoronaric bypass	Blood culture negative (3 November 2006)	Yes (erythromycin, imipenem)	3·7 (27 October 2006)	12·6 (29 October 2006)	9·2 (27 October 2006)	10·1 (29 October 2006)
IP240	Male (57)	25 January 2007	26 January 2007	Negative	Positive 120 h (5 days) (31 January 2007)	*P. acnes*	Female (67)	Infectious endocarditis (*Enterococcus faecalis*), aortic and mitral valve replacement	Blood cultures negative (31 January 2007), tracheal secretion: *Klebsiella pneumoniae, Candida albicans*	Yes (vancomycin, imipenem)	3·93 (23 January 2006)	9·71 (27 January 2006)	18·6 (23 January 2006)	9·9 (27 January 2006)
IP816	Female (43)	20 March 2007	21 March 2007	Negative	Positive 132 h (6 days) (27 March 2007)	*P. acnes*	Male (47)	Pericardial lysis, aortic plastic valvular prosthesis aortocoronaric bypass	No microbiological Examination	Yes (cefazolin, clarithromycin)	0·49 (16 March 2007)	NT	8·0 (16 March 2007)	14·3 (22 March 2006)
IP095	Male (31)	29 June 2007	2 July 2007	Negative	Positive 116 h (5 days) (7 July 2007)	*P. acnes*	Male (74)	Ischemic cardiomyopathy, mitral valve replacement, aortocoronaric bypass	Blood culture (4 July 2007): *S. epidermidis*, tracheal secretion: *P. aeruginosa*	Yes (cefazolin, clarithromycin)	0·13 (29 June 2007)	4·88 (2 July 2006)	5·8 (28 June 2007)	13·2 (2 July 2007)

[a]TF, transfusion of platelet concentrate.

[b]Culture detection, detection time after sampling 24 h after donation.

[c]CRP, C-reactive protein (reference range ≤ 5 mg/l).

[d]Negative, negative after 7-day storage.