

Risk of piperacillin-induced hemolytic anemia in patients with cystic fibrosis and antipseudomonal treatment: a prospective observational study

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BACKGROUND: Drug-induced immune hemolytic anemia (DIIHA) is a rare but severe side effect caused by numerous drugs. Case reports and case series suggest that piperacillin-related DIIHA may be more common among patients with cystic fibrosis (CF). However, the prevalence is speculative. The aim of this prospective, observational study was to determine the prevalence of DIIHA in such affected patients.

METHODS AND MATERIALS: Patients with CF hospitalized for parenteral antibiotic therapy at Charité Universitätsmedizin Berlin, who had previously been exposed to IV antibiotics, were enrolled. Blood samples were collected on Days 3 and 12 of antibiotic treatment courses. Serological studies were performed using standard techniques with gel cards. Screening for drug-dependent antibodies (ddab) was performed in the presence of the drugs and their urinary metabolites.

RESULTS: A total of 52 parenteral antibiotic cycles in 43 patients were investigated. Ddab against piperacillin were detected in two patients (4.7%). The direct AHG was positive with anti-IgG only in both patients. However, only one of these patients developed mild immune hemolytic anemia. Both patients had been repeatedly treated with piperacillin without any evident hemolysis. There was no correlation between the exposure to piperacillin and the prevalence of ddab.

CONCLUSION: Our prospective study indicates that piperacillin-induced ddab occur more frequently in patients with CF than previously suggested. The question related to the significance of piperacillin-dependent antibodies may reflect new aspects in this field.

With an incidence rate of 1-4 per 1,000,000 individuals annually, drug-induced immune hemolytic anemia (DIIHA) is a rare but severe side effect of drug administration.^{1,2} Yet, DIIHA is likely underdiagnosed, as symptoms vary and hemolysis can be overlooked or misdiagnosed.³

More than 130 drugs have been reported to cause DIIHA. The most common causative agents are second- and third-generation cephalosporins, piperacillin, and platinum-based chemotherapies.⁴ The exact pathomechanism of DIIHA is unknown. One hypothesis is that the immune response to drugs may be initiated by the interaction of the drug or its metabolites with the target cell membrane, leading to the formation of neoantigens and consequently the production of drug-dependent antibodies (ddab) and/or autoantibodies (aab).^{1,5-7} Ddab react only in the presence of the drug and/or its metabolites and can be classified into two types. Most ddab of the first type belong to the IgG and/or IgM class and cause

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intravascular hemolysis via complement activation (so-called “immune-complex mechanism”). The vast majority of the second type belong to non-complement activating IgG antibodies (ab), such as penicillin-dependent ab, and cause extravascular hemolysis. In contrast, drug-induced aab react with target cells independent of the drug. They are neither clinically nor serologically distinguishable from idiopathic aab and usually cause extravascular hemolysis.⁷⁻⁹ In addition, some drugs can cause a positive DAT with or without hemolysis by nonimmunologic protein adsorption (NIPA).^{4,10}

Very little attention has been paid to the proportion of patients producing ddab and/or a positive DAT with or without hemolytic anemia. In studies dating back to the 1960s, approximately 3% to 5% of patients receiving large doses of IV (intravenous) penicillin and up to 30% of patients ingesting alpha-methyldopa for more than 3 months develop a positive DAT. However, only a small percentage of such patients develop penicillin-dependent or autoimmune IgG-mediated extravascular hemolysis, respectively.^{1,11-14} More recently, Quillen et al. reported on the prevalence of ceftriaxone-induced antibodies in patients with sickle cell disease (SCD) and HIV.¹⁵ To our knowledge, there are no other studies dealing with the prevalence of drug-induced antibodies.

Patients with cystic fibrosis (CF) suffer from chronic bronchial infections with multi-resistant, primarily gram-negative bacteria and require repeated treatment with high-dose antibiotics.¹⁶ Previous case reports¹⁷⁻²⁶ and case series^{27,28} suggest a higher incidence of DIIHA due to piperacillin in patients with CF compared to the general patient population. Indeed, more than 50% of the reported cases of piperacillin-induced hemolytic anemia (PIHA) were observed in patients with CF.²⁹⁻³⁶ PIHA is usually characterized by acute intravascular hemolysis due to complement activation.^{17,18,20,22,23,27,34,35,37} We recently reported a mild course of hemolytic anemia due to piperacillin/tazobactam treatment in a patient with CF.³⁸

The prevalence of drug-induced ab with hemolytic potential in patients with CF is unknown. This prospective study was conducted to evaluate the incidence of DIIHA in patients with CF and to assess the prevalence of ddab to RBCs as a possible risk factor or biomarker for DIIHA. The focus was on all relevant antipseudomonal antibiotics to evaluate their potential in causing DIIHA.

METHODS

Setting and study population

This prospective observational study was conducted at the Charité Universitätsmedizin Berlin. Patients at the CF center of the Charité (Pediatric Pneumology Department) were selected to represent patients with extremely high exposure to parenteral antibiotics.¹⁶ From this cohort of currently treated 450 patients with CF (320 adults, 130 children), pediatric (minimum age of 12 years) and adult patients

were recruited prospectively at the beginning of in-hospital IV antibiotic treatment courses between October 2015 and June 2016. Inclusion criteria were at least three previous parenteral antibiotic treatment courses and willingness to consent. Exclusion criteria were a history of organ transplantation. Patients were excluded from the final analysis in the case of an incomplete (<10 days of IV antibiotics) treatment course or incomplete laboratory analysis. The project was approved by the ethics committee of Charité Universitätsmedizin Berlin (Number: EA2/079/15).

Study design

After providing written informed consent, patients were interviewed using a standard questionnaire for drug allergies (ENDA Questionnaire³⁹), which is a structured questionnaire that evaluates drug allergy history. The following clinical information was additionally collected for risk factor analysis: FEV1 predicted (forced expiratory volume in 1 second, FEV1), CF transmembrane regulator (CFTR) genotype, microbial colonization, and a detailed quantitative history of exposure to IV antibiotics. Medical records were reviewed to expand and verify the information from the interviews.

According to standard protocols, treatment courses for patients with CF comprised two IV antibiotics for 14 days.⁴⁰ For included patients, testing for ddab was performed for all parenteral antibiotics administered during the respective treatment course (aztreonam, ceftazidime, cefepime, meropenem, imipenem, tobramycin, fosfomycin, colistin, tigecycline, ampicillin/sulbactam, and piperacillin/tazobactam). Additionally, testing for ddab against piperacillin/tazobactam was performed for all patients independently from the current treatment regime. EDTA samples were collected on Days 3 (+1) and 12 (+/- 2) of treatment and analyzed within 48-72 hours. Samples were stored at 2-8°C until further analysis. On Day 3, samples from 24-hour urine collections were taken for testing of early-onset and late-onset drug metabolites. Hemoglobin and laboratory evidence of hemolysis were investigated on Days 0, 3, and 12 of the treatment courses, using the following parameters: reticulocyte count, lactate dehydrogenase, and haptoglobin. In patients with verified ddab, follow-up samples for the investigation of hemolysis and serological demonstration of antibodies against RBCs were performed up to 6 months after the last antibiotic exposure, depending on patient availability.

Serological investigation

RBC antibodies were assessed by direct and indirect anti-globulin test (DAT and IAT, respectively) using standard techniques with gel cards (Bio-Rad, Cressier sur Morat, Switzerland). Antibody screening was performed with papain-treated RBCs on neutral cards in all patients with positive DAT and/or positive IAT. Monospecific DAT was conducted using DC-Screening I cards (Bio-Rad). Eluates

from patients' RBCs were prepared using the acid technique (BAG Health Care).

Ddab were investigated using the gel technique with polyspecific antiglobulin reagents (ID-Card LISS/Coombs, Bio-Rad), as previously described, with few modifications.^{41,42} Briefly, drugs were dissolved in 0.9% NaCl to prepare a 1 mg/mL drug solution. Plasma samples (25 µL) from patients were incubated for 30 minutes at 37°C with pooled group 0 RBCs (50 µL of a 1% vol/vol suspension) in the presence of the drugs (25 µL) and with different urinary (ex vivo) metabolites of the drugs (50 µL). To obtain early- and late-onset drug metabolites, samples from 24-hour urines (autologous urine) and from at least one different CF patient taking the same antibiotics were used as described above. In parallel, identical tests were performed using saline instead of the drug (Negative control 1). In addition, serum samples from random donors were incubated with RBCs in the presence of the drug and the metabolites, respectively (Negative control 2). In cases of a positive test for ddab in the presence of piperacillin/tazobactam (drug solution and/or ex vivo metabolites), the test was repeated using piperacillin and tazobactam as a single substance. In patients with a positive IAT before drug analysis, serum samples were dialyzed prior to analysis to eliminate potential drug residues and retested in the presence of the culprit drug.⁴²

RESULTS

Of 58 patients that provided informed consent, 43 patients fulfilled the criteria for analysis. Of these, six patients received more than one course of antibiotics. In total, 52 antibiotic cycles were analyzed. The following antibiotics were administered (number of patients treated): piperacillin/tazobactam (20), tobramycin (19), colistin (15), ceftazidime (13),

meropenem (11), fosfomycin (7), aztreonam (2), tigecycline (1), ampicillin/sulbactam (1), cefepime (1), and imipenem (1).

In 33 (63%) of the cycles, a positive IgG-DAT was detectable during antibiotic treatment. A newly positive DAT with IgG or an increase of RBC bound IgG was detectable in 26 (50%) of the cycles (Day 12 [+/- 2] vs. Day 3 [+1] of antibiotic treatment). Piperacillin/tazobactam and/or ceftazidime were involved in 24 of these 26 antibiotic cycles. In the subgroup of patients receiving piperacillin/tazobactam, 39% had a positive DAT on Day 3 of the treatment course, and 83% had a positive DAT at the end of the treatment course. Ddab against piperacillin were detected in two patients.

Patient 1

The first patient was a 50-year-old female with previous exposure to piperacillin without any evident hemolytic episode. During the study, she was treated with piperacillin/tazobactam for 14 days. On Day 3 of treatment, the patient had a weak positive DAT with IgG and weak positive antibody screening with papain-treated RBCs only. Drug-dependent antibody testing was weak positive with piperacillin/tazobactam and two ex vivo metabolites (including autologous patient urine). On Day 12 of antibiotic treatment, serological reactivity (DAT and IAT) was markedly increased and an autoantibody with Rhesus-e specificity (auto-anti-e) was detectable in the patient's plasma. After plasma dialysis, the autoantibody disappeared, signaling that the culprit drug had still been present in the patient's plasma. However, its reactivity was restored in the presence of piperacillin/tazobactam and five different ex vivo metabolites. This serological reactivity is typical for a drug-dependent antibody, but not for an alloantibody. Interestingly, 2 weeks after discontinuation of the drug, piperacillin-dependent antibody reactivity increased and specific ddab were detectable up to 7 months later (Table 1). No ddab against tazobactam or colistin were detected.

TABLE 1. Serological findings in two patients with piperacillin-dependent antibodies

Patient No.	Day	Ab screening		DAT			Piperacillin/Tazobactam-dependent ab			
		IAT	Papain	Poly	IgG	C3d	Eluate	Drug*	Ex vivo (au) [†]	Ex vivo (allo) [‡]
1	3	0	+++	++	++	0	0	+/-	+	+
	12	++	++++	+++	+++	0	0	+	++	++
	26	0	+	0	+/-	0	0	+++	+++	+++
	210	0	nt	0	0	0	0	+/-	+/-	0
2	3	0	nt	0	nt	nt	nt	0	0	0
	12	0	0	+/-	+/-	0	0	0	0	+
	15	0	nt	+	+/-	0	0	+	+	+++
	20	0	nt	0	0	0	0	++	+++	+++
	22	0	nt	0	0	0	0	+++	+++	+++
	100	0	nt	0	+/-	0	0	0	0	0

Treatment with piperacillin/tazobactam 3 × 4.5 g/d: Patient number 1 from Day 1 to Day 14; Patient number 2 from Day 1 to Day 12.
 Degree of reactivity: 0 (negative), +/- (very weak), + (weak), ++ (moderate), +++ (strong) ++++ (very strong).
 * Drug: piperacillin/tazobactam or piperacillin.
 † Ex vivo (au): autologous urine.
 ‡ Ex vivo (allo): urine from other patients taking the same drug (maximum degree of reactivity: in Patient number 1 urine from up to five different patients was tested after dialysis of the plasma; in Patient number 2 urine from up to four different patients was tested).
 Ab = antibodies; nt = not tested; IAT = indirect antiglobulin test; DAT = direct antiglobulin test.

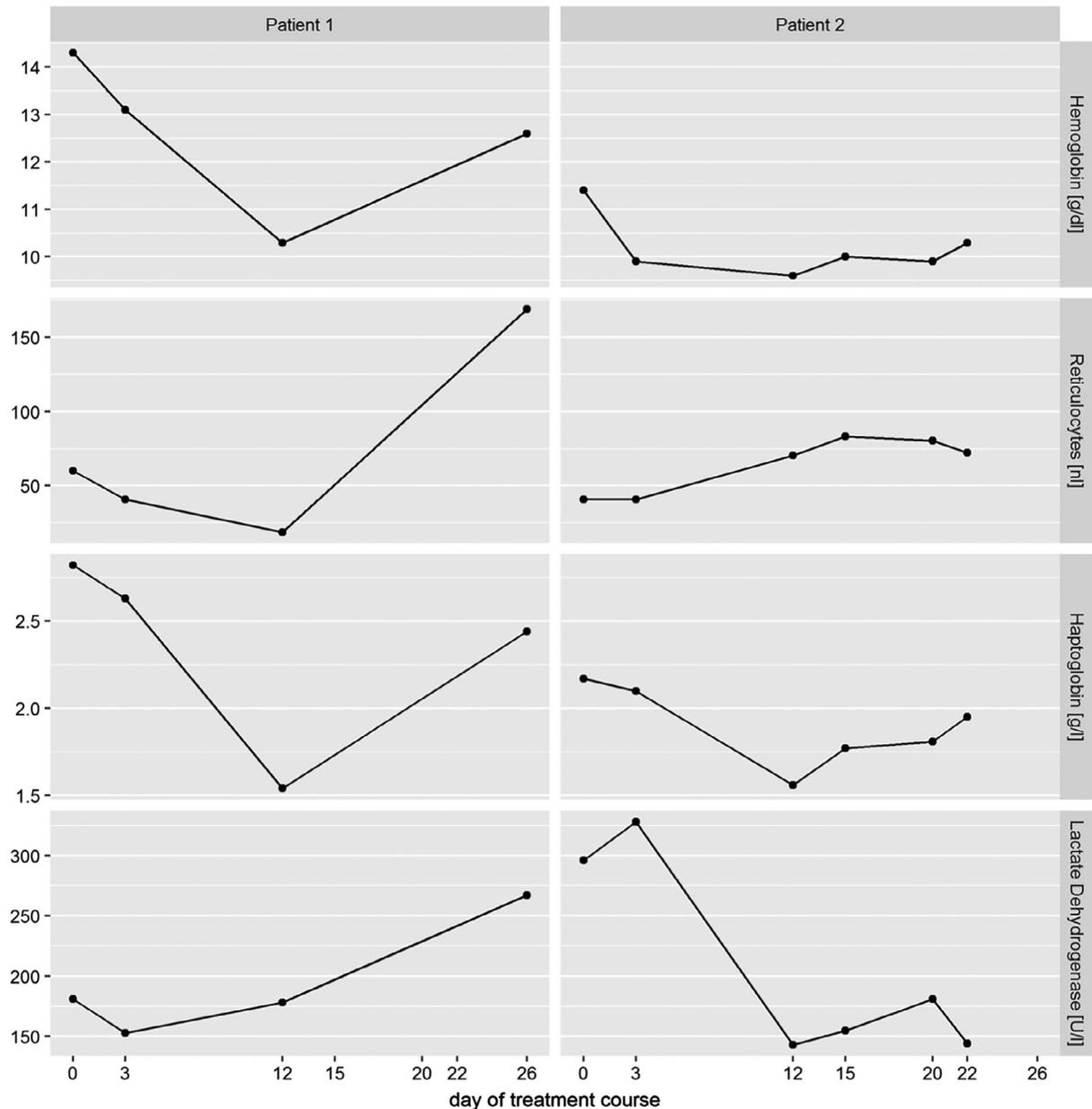


Fig. 1. The course of laboratory parameters in the two patients with piperacillin-dependent antibodies.

Despite a marked decrease in hemoglobin (from 14.3 g/dL on admission to 10.3 g/dL on Day 12) and detectable weak ddab on Day 3, continuous piperacillin therapy led to comparatively mild hemolysis. This was reflected by a mild increase in LDH and the reticulocyte count, which was only evident during follow-up (Day 26) but not during piperacillin treatment (Fig. 1).

Patient 2

The second patient was a 23-year-old female patient previously exposed to piperacillin without any evident hemolytic episode who had experienced urticaria once while receiving

piperacillin/tazobactam. She received three courses of antibiotics during the study. Initially, no ddab were detectable. On Day 12 of the second antibiotic course with piperacillin/tazobactam and tobramycin, the DAT became weakly positive with IgG. The antibody screening test was negative in the absence of any drug, but weakly positive in the presence of four different ex vivo metabolites of piperacillin/tazobactam. Follow-up samples on Days 3, 8, and 10 after completion of antibiotic therapy were also positive with a piperacillin/tazobactam drug solution and testing showed increasing reactivity during further observation. Three months later, ddab were undetectable (Table 1). Ddab against tazobactam or tobramycin were not detected.

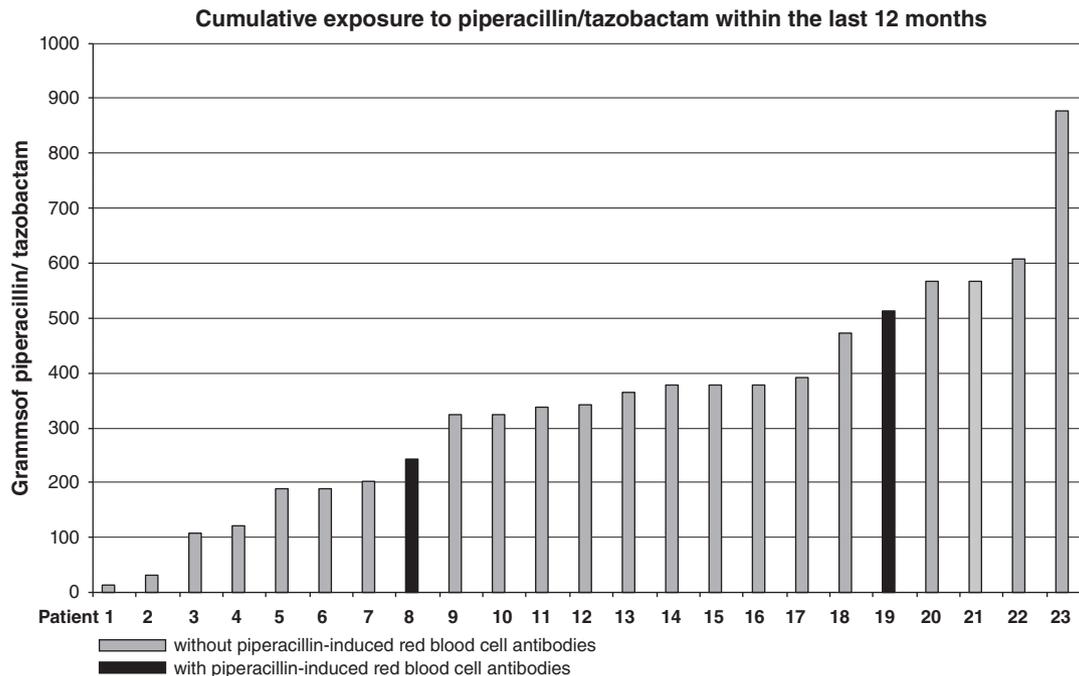


Fig. 2. Cumulative exposure to piperacillin/tazobactam within the last 12 months prior to the study in 23 patients. The two patients with detectable piperacillin-dependent antibodies are highlighted in dark gray.

Of note, the patient experienced a decrease in hemoglobin from 11.4 g/dL to 9.9 g/dL and mild to moderately elevated LDH of unknown reason from admission to Day 3. On Day 12, hemoglobin levels reached a nadir of 9.6 g/dL with normal LDH levels. Furthermore, haptoglobin levels and the reticulocyte count remained within normal limits throughout the observation time. Therefore, the patient did not show evidence of hemolysis at the time of the first serological detection of ddab to piperacillin (Day 12) (Fig. 1 and Table 1).

The usual dosage of piperacillin was 4.5 g three times per day over an average of 13.5 days in the patients without, and 13 days in the two patients with piperacillin-dependent antibodies. In the former case, the mean interval of the last piperacillin administration prior to the study was 4.6 months and in the latter case 7.5 months. The median number of previous piperacillin treatment courses within 30 months prior to study recruitment was not significantly different in patients with and without piperacillin-dependent antibodies (5.5 vs. 2 administrations). There was no correlation between the cumulative dose of piperacillin and the prevalence of piperacillin-dependent antibodies (Fig. 2). The prevalence of ddab against piperacillin was 4.7% (2 of 43) in the whole study, and 5.4% (2 of 37) in the subgroup of patients ever exposed to piperacillin or piperacillin/tazobactam. Ddab against other drugs were not detected. Risk factor analysis for FEV1, exposure to piperacillin/tazobactam, pseudomonal colonization, and total IgE levels were heterogeneous and did not reveal significant risks for DIIHA.

DISCUSSION

Drug hypersensitivity in patients with chronic bacterial infections and high antibiotic exposure is a common clinical problem. In the high-risk cohort of patients with CF, exanthema, drug fever, and bronchospasm have been reported to occur in up to 60% of treated patients.^{16,43–45} The present study reports on the first cross-sectional prospective trial to evaluate the incidence of DIIHA as a manifestation of drug hypersensitivity in patients with CF. The prevalence of 4.7% of piperacillin-dependent antibodies to RBCs shows that this phenomenon is more common than previously suggested. Our results are supported by previous research focusing on drug hypersensitivity in CF, which consistently shows piperacillin as the most immunogenic drug.^{46,47} Piperacillin appears to have a higher inclination of causing DIIHA than other antipseudomonal antibiotics. With the exception of one suspected case of ceftazidime-induced hemolytic anemia,⁴⁸ only piperacillin and piperacillin-tazobactam have so far been reported to cause DIIHA in patients with CF. These findings raise questions regarding whether PIHA is much more common in the high-risk cohort of patients with CF or in general, with mild manifestations potentially escaping detection. A study of a non-CF cohort with high exposure to antipseudomonal antibiotics, especially piperacillin, would be required to address these questions.

The onset and the severity of PIHA are variable and may occur within 1 to 14 days after initiating piperacillin or

piperacillin/tazobactam therapy. Hemoglobin frequently decreases to 3-5 g/dL^{18-24,26-28,31,33} or even lower levels^{25,37} and results in fatalities in up to 6% of cases.⁴⁹ Therefore, PIHA has been regarded as a highly acute and life-threatening event, particularly if it remains undetected in due time. In contrast, the two patients described in the present study and in a patient from a previously published case report developed only mild PIHA.³⁸

There is little information about the immunologic mechanism leading to the production of drug-dependent antibodies. In a recent study, drug-specific B cells were characterized. In piperacillin-hypersensitive patients with CF, drug-responsive B cells secreting drug-specific IgG were detected.⁴⁶ The significance of these IgG ab remains unclear. A subsequent study focused on the specificity of anti-piperacillin IgG ab using structurally-related drug-protein adducts. Anti-piperacillin IgG was shown to bind with high specificity to piperacillin.⁵⁰ However, the hemolytic potential of these antibodies has not yet been evaluated.

It remains unclear whether patients with CF are at a greater risk of developing piperacillin-dependent ab to RBCs simply due to high antibiotic exposure or if other predisposing factors exist. Descriptive analysis of risk factors in our study did not reveal trends for FEV1, total serum IgE, or cumulative exposure to specific antibiotics. The presence of an association between hypersensitivity reactions to antibiotics and drug-induced hemolysis of RBCs remains ambiguous. Patient number 2 had urticaria and our previous patient (described in the case report by Meinus et al.³⁸) had drug fever due to piperacillin before developing PIHA. In these two cases, a sequence of different immune responses to piperacillin could be observed. In our patient cohort with and without detectable piperacillin-dependent antibodies, there was no difference in the occurrence of previous allergic drug reactions to piperacillin. The only risk factors presently identified are piperacillin exposure and the diagnosis of CF.

While the cumulative dose of single antibiotics has been shown to be a risk factor for general drug hypersensitivity in patients with CF,¹⁶ an association between the administered drug dose and the occurrence of DIIHA is not likely.⁸ This is also supported by the results in the present study. Furthermore, repeated exposure to the same drug has been suggested to be important in the antibody formation process and DIIHA development.^{8,31,49,51} The latter findings are in line with the results of this study. Both of the patients described with PIHA were treated recurrently with piperacillin.

Another important issue is a potential predisposition between CF-related cofactors and the development of ddab and DIIHA. Altered CF transmembrane conductance regulator (CFTR) in CF patients has been hypothesized to potentially play an important role. The mutation in CFTR causes decreased nitrous oxide production, leading to vasoconstriction and an enhanced risk of intravascular RBC sludging. This may explain why patients with CF are more susceptible

to severe forms of DIIHA.²⁰ The chronic hyperinflammatory state in CF patients may also be crucial,^{19,52} as inflammation is associated with a higher probability of alloimmunization. This might be comparable to the alloimmunization process of transfused RBC, whereby an increased inflammatory status of the recipient appears to enhance the immune response to transfused antigens.⁵³

Whether patients with mild hemolysis can have a sequence to severe intravascular hemolysis with complement activation under repetitive treatment with the culprit drug is an open question.^{54,55} There is some evidence that the complete clinical picture of PIHA in patients with CF might be preceded by a mild hemolytic episode, which may be overlooked in some cases.²⁶ In Garcia, Gala and colleagues report on a patient with immune hemolysis due to piperacillin/tazobactam, the patient developed weak positive DAT, hemolysis, and increased serological reactivity under continuous treatment with the drug.²⁹ This is in line with the clinical course of Patient number 1, in which the serological picture changed remarkably under continuous treatment with piperacillin/tazobactam. The patient eventually showed a strong positive DAT, negative eluate (without adding piperacillin), and detectable auto-anti-e antibodies. In addition, there was laboratory evidence of hemolysis.

The significance of ddab and/or a positive DAT without clinical or laboratory signs of hemolysis is rather speculative. Ceftriaxone-induced RBC antibodies have been reported to be common in children with SCD or human immunodeficiency virus (HIV) infection, with a high likelihood of ceftriaxone exposure. The reported prevalence was 5.3 and 15.6%, respectively. Only two of the eight patients with detectable ddab developed hemolysis, with one fatal case.¹⁵ As ceftriaxone is rarely used in patients with CF due to its poor antipseudomonal activity, there are no equivalent data concerning the prevalence of ceftriaxone-induced antibodies in the CF patient group.

The pathomechanism may be somewhat comparable to that in patients receiving large doses of penicillin or alpha-methyl dopa, where only a proportion of patients that develop a positive DAT and/or aab develop DIIHA or autoimmune hemolysis, respectively.⁸ In patients with warm reactive aab of IgG type, the ability to activate complement has been proposed as one of the variables that predict the occurrence of severe hemolysis.^{1,56} Most of the reported cases of PIHA implicate a complement-mediated intravascular process. Only a few cases with severe hemolysis without detectable complement fragments on patient RBC have been described.^{25,33,34} The observation that both of the patients with piperacillin-dependent ab in our study did not develop severe or significant hemolysis may be explained by the occurrence of non-complement activating IgG ab. Further studies are required to determine predictive factors to identify patients with detectable ddab at risk of developing DIIHA.

The clinical significance of the frequently observed positive DAT under antibiotic treatment in our study remains

unclear. Penicillin and cephalosporin administered in large doses can frequently lead to a positive DAT and hemolysis in a few cases. Some drugs are able to alter the RBC membrane, leading to protein (e.g., immunoglobulins) uptake and as a result to positive DAT in immunohematology testing, the so called “nonimmunological protein adsorption (NIPA).”^{4,10} Therefore, NIPA is independent of the drug-antibody formation. NIPA is known to occur as a result of the tazobactam but not the piperacillin component of piperacillin/tazobactam.⁵⁷ The high rate of positive DATs in patients treated with ceftazidime was unexpected, as this has only been described previously in other cephalosporin’s, e.g., cefotetan, cephaloridine, and cephalothin.⁴

In summary, the repetitive use of piperacillin can lead to piperacillin-dependent antibodies in a certain number of patients (with CF) and subsequently lead to clinically relevant PIHA in a portion of these patients. The predictive value of ddab detection per se and potential predisposing factors for developing severe hemolysis in these patients need to be addressed in future studies.

Strengths and limitations

To the best of our knowledge, this study is the first prospective study on DIIHA in patients with CF. To date, DIIHA has been regarded as a rare complication of drug treatment. Despite the limited number of patients enrolled, our data suggest a higher prevalence of piperacillin-induced antibodies in the high-risk cohort of patients with CF and suggest a new recommendation for patient safety in high-risk cohorts.

A potential bias lies in patient selection toward patients with advanced lung disease because of their higher need for parenteral antipseudomonal treatment and the exclusive recruitment of patients from the inpatient ward. Such patients frequently have high levels of inflammation that may favor adverse reactions. Therefore, the transferability of the high incidence of DIIHA in this study to other cohorts with lower levels of inflammation may be limited.

A limitation of our study might have been that we used plasma but no serum samples. Some ddab activate complement. In these cases, the use of serum might be better than the use of plasma. Furthermore, we used drug solution for testing but no drug coated RBCs. For the investigation of piperacillin ddab, testing in the presence of the drug is the more reliable method compared to using drug coated RBCs.⁵⁸ However, in the samples which were negative in our study, we could have missed ddab against other antipseudomonal antibiotics only reacting with drug coated RBCs. Therefore the prevalence of ddab in our study might have been underestimated.

Finally, the present study has provided insight on an important issue for the safety of patients with CF and all patients with chronic exposure to piperacillin. Following the detection of drug-dependent hemolytic antibodies, the only recommendation that can be presently made is the life-long

avoidance of the culprit antibiotics. Furthermore, as a positive DAT is known to be a necessary but insufficient condition for hemolysis, we suggest screening patients with repeated and/or high exposure to piperacillin for a positive DAT. Patients that develop a positive DAT under piperacillin treatment should be closely monitored for hemolysis and/or screened for the presence of piperacillin-dependent ab.

Our findings should be confirmed in a multicenter prospective trial to confirm the high incidence of DIIHA in patients with CF and in other high-risk patient cohorts. Additionally, risk factor analysis in a larger cohort may reveal new findings.

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CONFLICT OF INTEREST

The authors declare to have no conflict of interests.

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