Diffuse alveolar hemorrhage (DAH) is a life-threatening syndrome that complicates a large number of clinical conditions in critically ill children. Although the incidence of DAH is difficult to estimate, DAH was the principle cause of death in about 9% of neonatal autopsies. When mechanical ventilator support was required, DAH mortality exceeded 50% in immunocompromised patients. The therapeutic options for acute intervention in DAH are limited and frequently unsuccessful.

Tranexamic acid (TXA), a synthetic derivative of the amino acid lysine, is an anti-fibrinolytic agent that exerts its action through binding to plasminogen preventing its binding to fibrin and hence its activation to plasmin. Systemic and local TXA administration has long been used for treatment and/or prophylaxis of bleeding episodes in patients with congenital and acquired bleedings. Several studies have suggested superior effectiveness of local versus systemic administration of TXA. TXA mouthwash was superior in reducing rates of postoperative bleeding in anticoagulated patients undergoing dental surgery and in controlling spontaneous gingival bleeding in hemophilia patients. TXA was also successful in controlling bleeding when

**Pilot trial of a novel two-step therapy protocol using nebulized tranexamic acid and recombinant factor VIIa in children with intractable diffuse alveolar hemorrhage**

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**BACKGROUND AND OBJECTIVES:** Diffuse alveolar hemorrhage (DAH) is a life threatening condition with very limited, often unsuccessful, therapeutic options. This study aimed at exploring the feasibility and efficacy of nebulized tranexamic acid TXA (n-TXA) and nebulized recombinant factor VIIa (n-rFVIIa) when used in a two-step therapy protocol in children with intractable DAH in a pediatric intensive care unit.

**METHODS:** In a prospective trial, n-TXA (250 mg/dose for children <25 kg and 500 mg/dose for children >25 kg) was administered to 18 children (median age [interquartile range]; 24.0 months [11.3, 58.5]) with intractable DAH. N-rFVIIa (35 µg/kg/dose for children <25 kg, and 50 µg/kg/dose for children >25 kg) was added if no or minimal response was seen after 3 to 4 doses (18 to 24 hours) of n-TXA.

**RESULTS:** DAH was stopped in 10 (55.6%) children with n-TXA alone within 24 hours of therapy. Documented concomitant respiratory infection showed a significant negative association with response to n-TXA in a step-wise regression analysis (OR=0.06; 95% CI=0.01–0.74). In the other 8 (44.4%) children, n-rFVIIa was added due to n-TXA failure. Six (75.0%) showed complete cessation of DAH, while two children failed to respond with the addition of n-rFVIIa (25.0%). None of the children who responded to therapy showed recurrence of DAH after therapy termination. No complications related to therapy were recorded.

**CONCLUSIONS:** n-TXA and n-rFVIIa were effective and safe when used in a two-step-therapy protocol to control intractable DAH in pediatric patients in intensive care settings. This therapy modality warrants further exploration through larger multicenter clinical trials.
directly administered intrapleurally and into the pericardial cavity.9

Recombinant factor VIIa (rFVIIa) is a hemostatic agent that achieves hemostasis by one of two mechanisms: 1) activating factors X and IX at sites of tissue injury through binding to tissue factor (TF) and activated platelets resulting in thrombin generation; 2) TF-independent mechanism, in which rFVIIa directly activates factor X on the surface of activated platelets.10 Although rFVIIa is licensed for the treatment and prevention of bleeding in patients with factor VII deficiency or hemophilia with factors VIII or IX inhibitors, there is an increasing interest in off-label use of intravenous rFVIIa in children with clinical bleeding due to nonhemophilic reasons,11 including DAH in newborns12 and children.13 Several case reports reported the successful use of locally administered rFVIIa to adults with DAH. With the exception of one case in which nebulization of rFVIIa was used after failure of intravenous rFVIIa, 14 direct intrabronchial instillation was the primary method of drug delivery in all reports.15-17 Recently, two case reports were published in which DAH was treated by direct intrabronchial rFVIIa instillation in children.18,19

DAH in most cases represents a form of an intra-alveolar coagulopathy that is predominantly mediated by intra-alveolar TF expression in response to severe lung injury and is associated with fibrin deposition and secondary fibrinolysis.15,20,21 This understanding of the pathophysiological basis of DAH makes it plausible to assume superior efficacy if TXA and rFVIIa were used in combination in severe forms of DAH. Such an interventional approach has not been tried before. The current study aimed at exploring the feasibility and efficacy of a novel two-step therapy protocol using nebulized TXA (n-TXA) followed by nebulized rFVIIa (n-rFVIIa) in critically ill children suffering from intractable DAH in a pediatric intensive care unit (PICU).

PATIENTS AND METHODS

Study setting and design

We conducted a prospective non-randomized pilot trial to test the applicability and effects of n-TXA and n-rFVIIa in critically ill children with intractable DAH. The study was conducted over 24 months from January 2012 to December 2013 in PICU, at Prince Sultan Military Hospital, Riyadh, Saudi Arabia. The protocol of the study was approved by the institutional review board. A waiver of informed patient consent was obtained, given that n-TXA and n-rFVIIa were compassionate treatments that were used as emergency intervention for a life-threatening condition after exhaustion of possible traditional therapeutic interventions.

Study population

We involved all PICU children who developed DAH due to various causative illnesses during the study period. All children were either ventilated prior to the development of DAH because of respiratory failure or as a postoperative measure; or were put on mechanical ventilation as part of managing severe hemoptysis while in PICU. To be eligible for inclusion, children had to show evidence of DAH judged by the treating physician as minimally or non-responsive to conventional therapies 12-24 hours after its detection. Such conventional therapies included bronchial tamponade through positive pressure mechanical ventilation and/or transfusion therapy as needed (platelets transfusion (10 mL/kg), fresh frozen plasma (10 mg/kg), packed red blood cells (15 mL/kg), cryoprecipitate (10 mL/kg). Excluded were children who had congenital hemorrhagic disorders, those with associated nonpulmonary bleeding and those who showed a terminal disseminated intravascular coagulopathy. During the study period 18 children were eligible for inclusion.

Case definition

The diagnosis of DAH was based on meeting all of the following criteria: (i) the continuous presence of pink or red frothy liquid drains in the endotracheal tube (ETT) aspirates confirmed by persistent of grossly bloody lavage on three sequential bronchoalveolar lavage (BAL) aliquots to exclude a gastrointestinal or upper airway bleedings with aspiration of blood into the lung; (ii) chest radiograph showing a unilateral or bilateral diffuse pulmonary infiltrates; (iii) increasing respiratory distress; and (iv) evidence of systemic deterioration such as shock. Clinically confirmed DAH is diagnosed when all criteria were met.22

Intervention and protocol

Study protocol is illustrated in Figure 1. Children eligible for the study received n-TXA via ETT using inspiratory phase activated jet nebulizer every 6 hours. Tranexamic acid injectable solution (Cyklokapron Inj, 100mg/ml, Octapharma, Sweden) was used for nebulization. In case of response, n-TXA was continued for 2-3 doses after bleeding completely stopped. If no or minimal response was seen after 3 to 4 doses of n-TXA (18 to 24 hours from the first dose of n-TXA) or if bleeding severity escalated as judged by the treating physician at
any time before that, rFVIIa (Novoseven RT, 1mg lyophilized powder vials, Novo Nordisk, Denmark) nebulization was added every 4 hours until bleeding stopped. Before each n-rFVIIa dose a judgment was made by the treating physician whether evidence of active DAH still existed or not. If not, n-rFVIIa was stopped and n-TXA continued for another 2-3 doses. The maximum duration of therapy allowed was three days. If bleeding did not stop by three days of total therapy or if it reemerged after temporary cessation, nebulization therapy was not pursued further.

The n-TXA was used at a dose of 250 mg/dose for children <25 kg and 500 mg/dose for children >25 kg. The n-rFVIIa was used at a dose of 35 µg/kg/dose added to 2 mL saline for children <25 kg, and 50 µg/kg/dose added to 3 mL saline for children >25 kg. rFVIIa was reconstituted according to manufacturer instructions. After the first dose of rFVIIa was drawn from a vial, the vial was stored in a refrigerator (at 2 to 8°C). For subsequent doses administrated within 24 hours the same vial was used. Any unused product at the end of 24 hours was disposed of in accordance with local requirements.

Apart from n-TXA and n-rFVIIa, treatment of studied children followed the unit protocols with no intervention from the side of the investigators. Blood product administration was continued during the study as indicated by the results of laboratory investigations (hemoglobin level, platelets count, prothrombin time (PT) and activated partial prothrombin time (PTT)). Blood samples were collected immediately before n-TXA administration for platelets count, PT and PTT.

Main Endpoints, Data Acquisition and Processing
A standardized data collection form was used to extract data from each child’s medical record (medical history, demographics, baseline diagnosis and proven respiratory infection by positive BAL or tracheal aspirate). Also, laboratory indices (platelets count, hemoglobin, PT and PTT) immediately before the administration of n-TXA were recorded. The degree of DAH was judged as mild/moderate or severe. Severity of DAH was judged using previously published consensus criteria.22 Accordingly, we considered DAH to be severe if the bleeding was accompanied by worsening of respiratory distress or respiratory failure, cardiovascular instability, and/or diffuse bilateral pulmonary infiltrates. Severity of illness and predicted death rate was assessed for each patient in his/her first 24 hours of PICU admission using the Pediatric Risk of Mortality III (PRISM III) score. For risk stratification, a PRISM III score ≥24 was used. This was shown to correspond to a predicted death rate of 50%.21

The primary outcome of this study was the clinical response to n-TXA and n-rFVIIa. The children were followed prospectively until death or discharge from the PICU. Clinical response was defined as cessation of or decrease in the pink/red aspirates coming from the ETT as well as a reduction in the required ventilatory support. The secondary objective of this study was to determine tolerability and complications of therapy including thromboembolic related incidents, convulsive seizures and worsening of gas exchange.

To examine for predictors of response to treatment, children were divided into two groups: responders and non-responders to n-TXA. Patient characteristics and laboratory indices were compared between the two groups to determine factors affecting their clinical response. In those who failed initial n-TXA alone, predictors of response could not be assessed statistically given the small number of children remaining. All patients, including the two who died were included in the analysis.

The n-TXA and n-rFVIIa in DAH

Figure 1. Study protocol and main outcomes of intervention. In the whole group 2 children (11.1%) died while in PICU and 16 children (88.9%) were discharged from PICU. All deaths were in the n-TXA non-responder group. The two cases that did not respond to n-FVIIa included a 2-year-old girl with hemophagocytic lymphohistiocytosis; and a 10-year-old boy with acute lymphoblastic leukemia. In both cases bleeding stopped in the following 24 hours after nebulization therapy was stopped. The boy with acute lymphoblastic leukemia died a week later in the course of septic shock and multiorgan failure with recurrence of DAH. The other death was an 11-year-old boy whose DAH was controlled after adding n-rFVIIa; he died 16 days later in the course of septic shock and refractory hypoxemia not responsive to treatment. DAH, diffuse alveolar hemorrhage; n-TXA, nebulized tranexamic acid; n-rFVIIa, nebulized recombinant factor VIIa.
Statistical analysis
Data were analyzed using SPSS version 16.0 statistical package. Rates of response to n-TXA and n-rFVIIa were calculated. Descriptive analysis was done for all variables. Unless otherwise indicated, continuous variables were expressed as mean ± standard error and/or median (interquartile range) and categorical variables as number (percentage). The use of SEM with the mean was to show the uncertainty around the estimate of the mean and how close the sample mean would be to the population mean. The data was not normally distributed and the sample size was small. Responders and non-responders to n-TXA were compared using the Mann–Whitney U test for independent continuous variables; and the Fisher exact test or Pearson chi-square for categorical variables, as appropriate. For these analyses, the level of significance was taken as a P value ≤.05. Stepwise logistic regression was used to examine for predictors of response to n-TXA. Using univariate analysis, the odds ratio (OR) and 95% confidence interval (CI) for the association of response to n-TXA with each variable alone was calculated. All variables with a high probability of predicting response were entered in a final predictive stepwise regression analysis.

RESULTS
Eighteen children received n-TXA for DAH during the study. Table 1 gives the baseline characteristics of the cohort. Clinically, DAH stopped completely or nearly stopped in ten (55.6%) children with n-TXA alone within 24 hours of therapy (responders group). The other 8 (44.4%) children showed no or minimal clinical improvement of DAH (non-responders group); and hence, n-rFVIIa was added to their nebulization therapy. In the responders group, bleeding stopped after a mean of 2.6 (1.3) n-TXA doses (range; 1-4) and 13.2 (2.6) hours (range; 2, 24) from initiation of therapy. The demographic, clinical and laboratory characteristics of responders and non-responders to n-TXA are compared in Table 2.

Table 3 shows the results of univariate logistic regression analyses of possible predictors of response to n-TXA. Documented respiratory infection showed a significant negative association with response to n-TXA (OR=0.06; 95% CI=0.01–0.74). Baseline cardiac diagnosis was associated with the highest probability of responding to n-TXA, although it did not reach significance. In a predictive stepwise regression analysis, a test of the full model against a constant only model was statistically significant, indicating that the predictors as a set reliably distinguished between responders and non-responders to n-TXA (chi square=6.48, P=.01 with df=1). In the stepwise regression analysis, documented respiratory infection remained the only significant factor associated with response to n-TXA where it was negatively associated with response to the drug (P value=.02).

Of the eight children who received n-rFVIIa, six (75.0%) showed complete cessation of DAH within
three days of nebulization therapy. DAH failed to stop in two children (25.0%) during the study period. The latter two cases included a 2-year-old girl with hemophagocytic lymphohistiocytosis and a 10-year-old boy with acute lymphoblastic leukemia. Both had documented associated respiratory infection. In both cases bleeding stopped in the 24 hours after nebulization therapy was held. The boy with acute lymphoblastic leukemia died a week later in the course of a septic shock and multiorgan failure with recurrence of DAH. The other death was an 11-year-old boy with mixed connective tissue disease admitted with severe respiratory failure and *Streptococcus pneumoniae* pneumonia. His DAH was controlled after adding n-rFVIIa. He died 16 days later in the course of a septic shock and refractory hypoxemia not responsive to mechanical ventilation.

In responders to n-rFVIIa, the mean time elapsed from application of n-TXA and n-rFVIIa to cessation of clinically overt DAH were 43.5 (3.7) hours (range: 34-54) and 16.0 (2.7) hours (range: 10-24), respectively; and the number of n-rFVIIa doses administered was 4.1 (0.6) per child (range: 3-6).

None of the children who responded to therapy showed recurrence of DAH after therapy was stopped, while still in the PICU. No complications related to therapy were recorded in any of the studied children. In the whole group, 2 children (11.1%) died while in PICU and 16 children (88.9%) were discharged from PICU. None of the deaths were attributable to side effects of the drugs used in this study (Figure 1).

**DISCUSSION**

We conducted an interventional clinical trial of TXA and rFVIIa nebulization in a group of severely ill children suffering from intractable DAH due to various causes in intensive care settings. In this trial, 10 of 18 (55.6%) children with intractable DAH responded to 1 to 4 doses of n-TXA within 2-24 hours of therapy. Another 6 children (33.3 %) responded to 3 to 6 doses of n-rFVIIa as a second step in therapy. All responders continued n-TXA for another 2–3 doses and did not show any therapy-related complications or DAH recurrence after cessation of therapy.

In as much as DAH is a life-threatening emergency with poor outcome, treatments available so far are empiric, nonspecific and with no proven efficacy. Possible reasons for this are the ambiguity of pathogenesis and the diversity of conditions associated with DAH. In such an emergency the priority should be given to local hemostasis. Combined n-TXA and n-rFVIIa in DAH has not been used before in children, but a few

### Table 2. Comparison of demographic, clinical and laboratory characteristics between n-TXA responder and non-responders.

| Demographics | Responders (n=10) | Non-responders (n=8) | P value |
|--------------|------------------|---------------------|---------|
| Age (mo)a    | 38.1 (12.9)      | 49.0 (18.5)         | 1.00    |
| Sex (M/F)    | 6/4              | 4/4                 | 1.00    |
| Weight (kg)a | 14.1 (3.3)       | 17.1 (5.5)          | .85     |
| Baseline diagnosis | Connective tissue diseaseb | 3 (30.0) | 3 (37.5) | .58 |
| Cardiacb     | 4 (40.0)         | 1 (12.5)            |        |
| Malignancyb  | 1 (20.0)         | 2 (25.0)            |        |
| Documented respiratory infection | Yesb | 3 (30.0) | 7 (87.5) | .02 |
| No           | 7 (70.0)         | 1 (12.5)            |        |
| DAH severity | Severeb | 6 (60.0) | 4 (50.0) | 1.00 |
| Mild/moderateb | 4 (40.0) | 4 (50.0) | |
| Laboratory data | Platelets count (×10⁹/L)a | 204 (45) | 201 (63) | .82 |
| Hemoglobin (gm/dLa) | 10.3 (1.1) | 9.6 (0.8) | .64 |
| INRb | 1.26 (0.10) | 1.23 (0.11) | .89 |
| PT (sec.)b | 14.2 (1.2) | 13.6 (1.7) | .56 |
| PTT (sec.)b | 43.2 (5.0) | 46.6 (5.3) | .59 |
| First 24h PRISM III scorea | 24.20 (2.30) | 28.37 (2.37) | .26 |
| Predicted death rate on admission (%)a | 52.6 (8.9) | 67.1 (8.8) | .26 |
| PICU length of stay (days)b | 22.7 (5.4) | 21.4 (3.5) | .62 |
| Outcome | Discharged from PICUb | 10 (100) | 6 (75.0) | .18 |
| Died in PICUb | 0 (0.0) | 2 (25.0) | |

*aMean value (SE), median (interquartile range); bNumber (%) of patients.*
Table 3. Association of responsiveness to n-TXA with demographic, clinical and laboratory characteristics in the study groups.

| Demographics                        | Responders (n=10) | Non-responders (n=8) | OR^a | 95%, CI |
|-------------------------------------|-------------------|----------------------|------|---------|
| **Age**                             |                   |                      |      |         |
| ≤12 mo                              | 3                 | 3                    | 0.71 | 0.1-5.11|
| >12 mo                              | 7                 | 5                    | 1.00 | Ref.    |
| **Sex**                             |                   |                      |      |         |
| Male                                | 6                 | 4                    | 1.50 | 0.23-9.79|
| Female                              | 4                 | 4                    | 1.00 | Ref.    |
| **Weight**                          |                   |                      |      |         |
| ≤10 kg                              | 4                 | 4                    | 0.66 | 0.10-4.35|
| >10 kg                              | 6                 | 4                    | 1.00 | Ref.    |
| **Clinical characteristics**        |                   |                      |      |         |
| Baseline diagnosis                  |                   |                      |      |         |
| Connective tissue diseases          | 3                 | 3                    | 1.00 | 0.08-12.55|
| Cardiac                             | 4                 | 1                    | 4.00 | 0.21-75.65|
| Malignancy                          | 1                 | 2                    | 0.50 | 0.02-11.08|
| Other                               | 2                 | 2                    | 1.00 | Ref.    |
| Documented respiratory infection    |                   |                      |      |         |
| Yes                                 | 3                 | 7                    | 0.06 | 0.01-0.74|
| No                                  | 7                 | 1                    | 1.00 | Ref.    |
| DAH severity                        |                   |                      |      |         |
| Severe                              | 6                 | 4                    | 1.50 | 0.23-9.79|
| Mild/moderate                       | 4                 | 4                    | 1.00 | Ref.    |
| Laboratory data                     |                   |                      |      |         |
| Thrombocytopenia (≤150×10^9/mm^3)   |                   |                      |      |         |
| Yes                                 | 5                 | 4                    | 1.00 | 0.15-6.42|
| No                                  | 5                 | 4                    | 1.00 | Ref.    |
| Prolonged PT (>14sec.)              |                   |                      |      |         |
| Yes                                 | 5                 | 4                    | 1.00 | 0.15-6.42|
| No                                  | 5                 | 4                    | 1.00 | Ref.    |
| Prolonged PTT (>35sec.)             |                   |                      |      |         |
| Yes                                 | 6                 | 5                    | 0.90 | 0.13-6.08|
| No                                  | 4                 | 3                    | 1.00 | Ref.    |
| PRISM III score ≥24                 |                   |                      |      |         |
| Yes                                 | 6                 | 6                    | 0.5  | 0.06-3.84|
| No                                  | 4                 | 2                    | 1.00 | Ref.    |

^aResult of univariate models
case series in adults have shown good responses to local instillation of either TXA or rFVIIa. In a group of six adult oncology patients with lung involvement, direct application of TXA controlled hemoptysis when the bleeding source was identifiable during bronchoscopy, while nebulized TXA for 1-7 days controlled the bleeding when the bleeding source could not be identified.\(^{34}\) Intrapulmonary administration of rFVIIa to six critically ill adults with acute DAH resulted in complete and sustained hemostasis in three after a single dose while the other three required repeated intrapulmonary administration of rFVIIa to obtain hemostasis.\(^{15}\)

Regardless of the underlying disease, alveolar inflammation leading to release of inflammatory cytokines have been implicated in the pathogenesis of DAH.\(^{29,30}\) Experimental studies have shown bidirectional intense interaction between inflammatory and coagulation pathways in the bronchoalveolar compartment.\(^{26}\) In a manner comparable to systemic coagulopathy in disseminated intravascular coagulopathy (DIC), it has been postulated that an intra-alveolar coagulopathy process dominated by extensive alveolar TF expression takes place in DAH.\(^{20,27}\) In support of this, high intra-alveolar concentrations of TF were demonstrated in inflammatory lung conditions.\(^{21}\) Also, a several-fold increase in molecular markers of thrombin was documented in BAL fluid after lung injury.\(^{28}\) Asakura et al., showed that the pathophysiology of TF-induced DIC was considerably different from other forms of DIC, e.g. lipopolysaccharide-induced DIC. TF-induced DIC was associated with enhanced fibrinolysis and less organ fibrin deposition.\(^{29}\) Furthermore, TXA administration blocked these enhanced fibrinolysis properties.\(^{30}\) Thus, in this study, we started with n-TXA assuming that there was enough fibrin formation with secondary fibrinolysis being the major problem. N-rFVIIa was added when it was apparent that the clotting process was not producing enough fibrin to stop the bleeding. Combining TXA and rFVIIa was shown to increase clot resistance to accelerated fibrinolysis through improving clot stability.\(^{15}\)

Although rFVIIa can act through a TF-independent mechanism, usually when administered in high doses, its principle mode of action is through interaction with TF resulting in local activation of factor X, which in our view is the most likely explanation for the alveolar hemostasis effect observed in this study. Nevertheless, some published experimental data may contradict this explanation. Tissue factor pathway inhibitor (TFPI), an anticoagulant protein primarily synthesized by endothelium, was shown to increase markedly in the alveolar space in patients with established lung injury.\(^{31}\) TFPI is a strong inhibitor of the TF-factor VIIa-dependent factor Xa generation,\(^{32}\) a fact that should have hindered the hemostatic action of n-rFVIIa in our patients. Also, TFPI was shown to interrupt activation of coagulation at other steps, including at the prothrombinase complex and thrombin generation.\(^{33}\) The majority of TFPI are secreted towards the alveolar space.\(^{34}\) Local administration of TXA and rFVIIa might have succeeded in achieving drug concentrations high enough to override the presumed effects of intra-alveolar TFPI. This conforms to reported observations that pulmonary hemostasis can be achieved more effectively from the air-side than from the systemic-side.\(^{15}\)

Thrombocytopenia or an abnormal coagulation profile did not influence the initial response to n-TXA in our patients. This observation agrees with previous reports on the use of TXA to prevent or treat bleeding.\(^{24}\) Associated respiratory infection was a predictor of poor response to TXA, and the two children who failed n-rFVIIa had documented respiratory infection. Infection could have shifted the intra-alveolar coagulopathy towards more coagulation inhibition. Experimental studies lend some evidence supporting this assumption. E. coli bacteria administered to baboons resulted in a progressive increase in the intra-alveolar levels of TFPI that interrupted activation of coagulation.\(^{35}\)

The safety profile of n-TXA and n-rFVIIa as used in this study was excellent. We did not notice any systemic or local side effects to therapy. The main feared systemic adverse events were thromboembolic-related incidents and seizures while worsening of gas exchange was the most feared local adverse event. This confirms previous studies that have shown no change in coagulation variables post intrabronchial instillation of TXA and rFVIIa.\(^{15,24}\) Also, the theoretical fear that such therapy might induce widespread alveolar fibrin deposition that would interfere with gas exchange\(^{15}\) was not substantiated by our results. The systemic use of TXA and rFVIIa, alone or in combination, was linked to an increased risk of serious thromboembolic complications.\(^{11,36,37}\) Also, local administration can achieve a higher concentration at the site of bleeding.\(^{38}\) Thus, we opted to use local administration of the drugs.

Nebulization was preferred to direct intrabronchial instillation in this study, since nebulization therapy offers a more uniform and homogeneous distribution of the drug dose along the distal airways. Experimental studies have shown that nebulized drugs achieve high concentrations in the more peripheral airways in ventilated animals, even in those with pneumonia.\(^{39}\) In a recent case report, in which DAH was treated by direct intrabronchial rFVIIa instillation in two-year-old boy,\(^{18}\)
treatment was withdrawn after a brief period of initial response due to worsening of bleeding accompanied by obstruction of the ETT with an extensive intraluminal blood clot. The authors warned that excessive clotting of the intraluminal blood present in the relatively small airways of children subsequent to rFVIIa administration may confer an increased risk of bronchial and ETT obstruction and could prevent rFVIIa from reaching the alveoli. We did not observe any complications with our approach using nebulization therapy of TXA or rFVIIa. During intrabronchial instillation, the drug dose is fractionated into 2-5 allocates and instilled; thus, a high concentration of the drug is achieved in the proximal airways and it is reasonable to expect rapid and strong generation of intraluminal fibrin clots in the proximal airways.

Two important limitations to this study should be considered. First, the study lacked a control group. However, nebulization of hemostatic agents was offered as a rescue treatment to children with uncontrollable life threatening DAH, in whom standard routine therapy for a sufficient period of time failed; and it would have been ethically and practically challenging to withhold therapy in a group of them to use as control. To avoid introducing bias as much as possible, inclusion criteria were sharply defined and by including all eligible cases of intractable DAH during the study period we were able to accurately describe the applicability and efficacy of the therapy protocol tested while drawing some powerful observations and hypotheses regarding predictors of response to treatment. Second, this is a single center study in a group of patients with heterogeneous diagnoses. The practice at our PICU may not be generalizable to other institutions. Also, the response to n-TXA and n-rFVIIa is likely to differ among background diagnoses and their specific therapy protocols. Despite these limitations, this study is the single largest trial that has reported the use of nebulization therapy in such an emergency situation. The novel approach of the two-step nebulization therapy (n-TXA followed by n-FVIIa) rests on a sound plausible pathophysiological background.

**CONCLUSION**

This preliminary clinical trial confirmed the feasibility of n-TXA and n-rFVIIa when used in a two-step-therapy protocol to control intractable DAH in PICU settings. While thrombocytopenia and an abnormal coagulation profile did not influence response to therapy, respiratory infection was a predictor of poor response. The two drugs as used in this study showed an excellent risk/benefit profile that warrants further exploration through a larger multicenter prospective double blind randomized clinical trial, possibly using a crossover protocol. A recommendation for use as a routine treatment for DAH cannot be made before the results of such trial are available.

**Conflict of interest**

The authors declare that there is no conflict of interest for this scientific work and that they received no funding for this research.
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