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Full Length Article

Detection of early incomplete heparin reversal following congenital cardiac surgery: A single-center retrospective observational study

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ABSTRACT

Background: The monitoring of unfractionated heparin (UFH) reversal with protamine plays a crucial role for bleeding management after cardio-pulmonary bypass (CPB) in congenital cardiac surgery. The current standard for the monitoring of UFH and its reversal is the activated clotting time (ACT). While the ACT is affected by other CPB-associated pathologies a bedside technique with more specific heparin-related results would be very helpful. The new point-of-care viscoelastic test Haemonetics TEG® 6s, which is based on small blood samples may fulfill these requirements. This study aimed to compare the new TEG with laboratory assays.

Methods: A retrospective observational study was performed on 40 children with a median age of 130 days (interquartile range 13 to 310 days) undergoing congenital cardiac surgery. After separation of CPB, test results of the TEG® 6s, ACT, anti-Xa for UFH and PTT were compared and correlated with each other.

Results: No clinically relevant correlation was found for heparin specific TEG-derived parameters (CK/CKH R-time ratio) with ACT, PTT and anti-Xa measurements. After grouping in dependence to the CK/CKH R-time in patients with and without successful heparin reversal again no significant difference of anti-Xa-UFH-levels, post-/pre-CPB ratio of the PTT and ACT was observed.

Conclusions: In pediatric patients undergoing cardiac surgery using CPB there is no association of conventional coagulation tests and TEG-derived results. While bedside viscoelastic tests deliver rapid results, further studies are needed to compare whether the TEG based management of incomplete heparin reversal is sufficient to monitor heparin reversal and to reduce blood loss.

1. Introduction

Heparin is the most commonly used drug for anticoagulation during cardio-pulmonary bypass (CPB) world-wide. It is administered either using weight-based dose calculation or individual calculation of patient-specific heparin concentrations. With the use of heparin during CPB safe anticoagulation and decreased bleeding can be achieved [1,2]. To reverse the heparin effect, protamine is routinely employed. The anticoagulatory heparin effect and its reversal are commonly monitored using the activated clotting time (ACT), which is rapidly available in the operating room. However, several studies have shown, that the ACT is not sufficient to detect incomplete heparin reversal [3]. Therefore, a technique that allows for fast detection of incomplete heparin reversal, would be very beneficial to decrease potential heparin-associated bleeding. Point-of-care (POC) viscoelastic tests using heparinase based assays offer this possibility [4]. Just recently a new single cartridge-based system using a resonance method was introduced into the clinical POC armamentarium (Haemonetics TEG® 6s system). This method allows for viscoelastic measurements out of 400 µl of citrated blood. This system may be especially valuable in pediatric cardiac surgery, where the amount and frequency of coagulation tests should be restricted due to small patient blood volumes.

This study aimed to compare the new viscoelastic coagulation testing system with conventional techniques to detect incomplete

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heparin reversal following protamine administration during pediatric cardiac surgery.

2. Methods

2.1. Patient selection and methods

Children up to 4 years (1460 days) of age undergoing congenital cardiac surgery using cardiopulmonary bypass (CPB) at our institution between 03/2018 and 09/2018 were retrospectively screened for potential inclusion in this study. Inclusion criteria were the use of the Haemonetics TEG® 6s system (Haemonetics Corporation, Braintree, USA) for viscoelastic measurements and timely corresponding routine laboratory coagulation analysis. For coagulation analyses two citrated blood samples were drawn at the same time point after CPB. One sample served for bedside thrombelastography (TEG) analysis and the other sample for analysis of conventional coagulation parameters, which were measured in the central laboratory of the university hospital in Tübingen, Germany. The TEG measurement was initiated within approximately 5 min after the sample was taken. Due to the transport time of the second citrated blood sample from the operating room to the central laboratory the conventional laboratory coagulation analysis was initiated within approximately 10 to 15 min after blood sampling. Patients were included when respective measurements had been performed perioperatively after heparin reversal with protamine. If repeated measurements had been performed only the first dataset of each patient was included into the analysis.

2.2. Coagulation management

All patients were managed for anticoagulation during CPB with individual heparin and protamine doses calculated with the Hepcon system (HMS Plus, Medtronic, Minneapolis, USA) with the goal to achieve an ACT above 450 s and a heparin level above 3.4 U/ml. If antithrombin III (ATIII) levels were below 75%, a single dose of 25E/kg ATIII was administered prior to the calculation of the heparin dose based on the institutional protocol. After heparin administration anticoagulation was monitored with repeated ACT measurements. During potential phases of hypothermia below 34 °C repeated heparin measurements were used to ensure a level above 3.4 U/ml. During the reperfusion phase before weaning of CPB an individual protamine dose was calculated using the Hepcon system. Successful heparin reversal was tested by ACT measurements and heparin-protamine titration assays 10 min after heparin reversal.

2.3. Coagulation analyses

According to our institutional protocol all children received a laboratory screening for coagulation disorders after separation from CPB 10 min after protamine administration including a viscoelastic assay, a blood count, Quick's value, partial thromboplastin time (PTT), anti-Xa levels for unfractionated heparin (UFH), fibrinogen, factor XIII, and ATIII levels. Anti-Xa UFH levels were measured using the INNOVANCE® Heparin assay (Siemens Healthineers, Erlangen, Germany).

2.4. Definition of incomplete heparin reversal

Thrombelastography with heparinase is discussed to be a sensitive method to detect circulating heparin. The R-time of the TEG measurements is defined as the time from the start of the analysis to first clot initiation (Fig. 1). The presence of heparin leads to a prolonged reaction time in the kaolin activated intrinsic pathway assay (TEG CK R-time), while additional heparinase should reverse this effect (TEG CKH R-time). A ratio of the kaolin activated assay to the assay with additional heparinase above 1 is considered to be suggestive for incomplete heparin reversal.

Additionally, the following ratios were calculated and a ratio above 1 is considered to be suggestive for incomplete heparin reversal:

- Ratio of the post-CPB to the pre-CPB PTT
- Ratio of the post-CPB to the pre-CPB ACT

2.5. Ethics approval

This study was approved by the Institutional Review Board of the University Hospital Tübingen (IRB#400/2018BO2).

2.6. Statistical analysis

Normal distribution of the variables was evaluated using skewness and kurtosis and the Kolmogorow-Smirnov test. Continuous variables are reported as either mean and standard deviation (mean ± SD) or medians and interquartile range (Median [IQR]) depending on the distribution of the data. Group comparisons of normally distributed variable were performed using Student's *t*-tests. Not normally distributed continuous variables were compared using the Wilcoxon Rank-Sum Test. Categorical variables are reported as percentages. Comparison of categorical variables was performed by the Fisher's exact test. Pearson's correlation coefficient with the calculation of 95% confidence intervals (95%CI) was used to identify the correlation coefficient. A Bland-Altman analysis including the calculation of the mean bias and the 95% limits of agreement (LOA) was performed for method comparison. A p-value of < 0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using the SAS JMP software (ver. 14.2.0, SAS Institute Inc., Cary, USA).

3. Results

During the study period 46 children up to 4 years of age undergoing congenital cardiac surgery were retrospectively screened for possible inclusion in our retrospective analysis. Four children were excluded because no TEG measurement had been performed during the perioperative course. Two additional children were excluded because no complete laboratory-based coagulation test was available. Finally, 40 children were included in the analysis. Demographics, types of surgeries, CPB parameters and temperature management of the included patients are given in Table 1.

No child was treated with low-molecular weight heparins or antiplatelet medication before surgery. Volume expanders like starch or gelatin derivates were not administered during surgery. In two datasets details regarding protamine dosing were missing. 27/38 (71.1%) of children received one protamine dose and 11/38 (28.9%) received a second protamine dose due to post-CPB coagulation analyses and clinical judgement. Conventional coagulation parameters and TEG results are given in Table 2. Intraoperative transfusion and coagulation factor application as well as postoperative bleeding rates are given in Supplement 1.

3.1. Correlation analyses

There was a weak negative correlation between the CK/CKH R-time ratio and the following parameters: ACT ratio ($r = -0.38$; 95%CI -0.643 to -0.036; $p = 0.0466$; Fig. 2a), PTT ratio ($r = -0.321$; 95%CI -0.578 to -0.006; Fig. 2b). No correlation could be observed between the CK/CKH R-time ratio and the anti-Xa UFH levels ($r = 0.002$; 95%CI -0.313 to 0.310; $p = 0.9928$; Fig. 2c). Although Bland-Altman analysis indicates a low mean bias of the agreement between CK/CKH R-time ratio, with the ACT ratio and the PTT ratio, the limits of agreement are considerably wide (right side of Fig. 2a and b). A weak positive correlation was observed between the anti-Xa UFH levels and the PTT ($r = 0.344$; 95%CI 0.036 to 0.592, $p = 0.0299$). The ACT levels did not correlate with the anti-Xa UFH levels ($r = 0.102$;

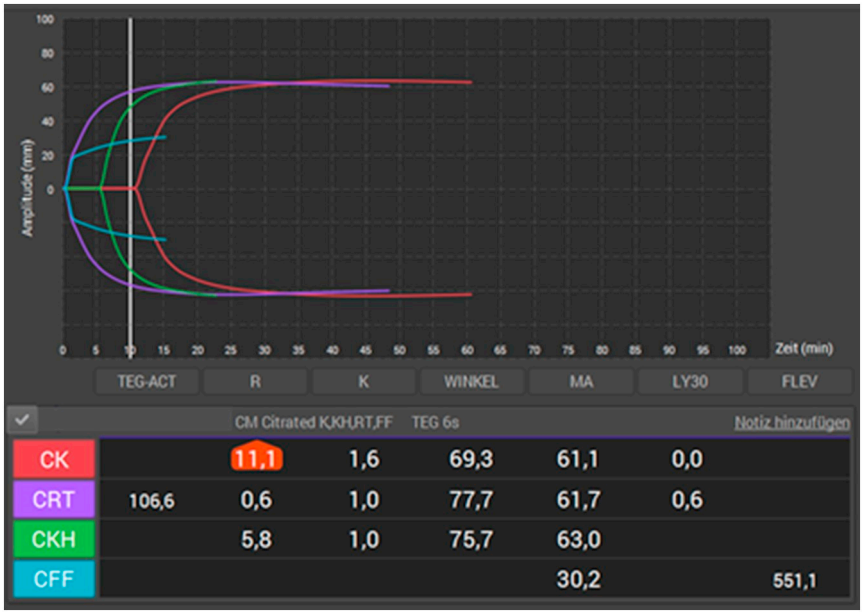

Fig. 1. Graphical result of the Haemonetics TEG® 6s system. R-time: time from start of the analysis to initial clot initiation. In this example a heparin effect is suggested as R-time of the CK test is prolonged and R-time of the CKH test is normal.

Table 1
Demographics and characteristics of included children.

Parameter	*N* = 40
Age (days)	130 (IQR 13 to 310)
Weight (kg)	5.5 (IQR 3.6 to 7.6)
Height (cm)	64 ± 14
STS classes	
Class 1	14 (35%)
Class 2	6 (15%)
Class 3	3 (7.5%)
Class 4	8 (20%)
Class 5	9 (22.5%)
Performed surgeries	
Aortic arch surgery	4
Norwood surgery	5
Arterial Switch	3
VSD repair	8
ASD repair	1
AVSD	2
Fallot surgery	5
BTS	2
Other	10
CPB	
Bypass time (min)	108 (IQR 80 to 165)
Clamp time (min)	62 (IQR 34 to 108)
Reperfusion (min)	13 (IQR 7 to 29)
Temperature (°C)	
Normothermia	6 (15%)
32–36 °C	24 (60%)
Below 32°	10 (25%)
Initial heparin dose (E/kg)	578 ± 167
Additional heparin during CPB (E/kg)	145 ± 134
Protamine dose (E/kg)	550 ± 179
Additional protamine dose after coagulation tests (E/kg) *N* = 11	99 ± 57
Total heparin to total protamine ratio	1.2 (IQR 1 to 1.5)

Results are given as Median [IQR] or Mean ± SD if normally distributed.
Abbreviations: STS: Society of Thoracic Surgeons, CPB: cardio-pulmonary bypass; VSD: ventricular septal defect; ASD: atrial septal defect; TVR: tricuspid valve repair; AVSD: atrio-ventricular septal defect; BTS: Blalock Taussig shunt.

Table 2
Conventional coagulation and selected whole blood count parameters and TEG® 6s results (of all included patients).

Conventional coagulation parameters at the time of the TEG® measurement		
Before cardiopulmonary bypass		
Hematocrit (%)		38.5 (IQR 33.7 to 43.8)
Platelets (x10^3/µl)		339 ± 153
Quick's Value (%)		81 ± 18
PTT (s)		29 (IQR 27 to 32)
Antithrombin-III (%)		85 ± 19
ACT (s) *n* = 32		134 ± 9
After cardiopulmonary bypass		
Hematocrit (%)		32.8 (IQR 30.5 to 34.9)
Platelets (x10^3/µl)		151 ± 62
Quick's Value (%)		64 ± 16
PTT (s)		31 (IQR 27 to 37)
Fibrinogen (mg/dl)		206 ± 56
Antithrombin-III (%)		86 ± 23
Factor XIII activity (%)		105 ± 28
Anti-Xa UFH (IE/ml)		0.15 (IQR 0 to 0.36)
ACT (s) n = 32		119 (IQR 104 to 127)
TEG® 6s measurements		
CK	R-time (min)	12 ± 5.8
	K-time (min)	2.4 (IQR 1.8 to 3.3)
	Angle (°)	62 (IQR 53 to 67)
	MA (mm)	52.2 (IQR 48.9 to 57.2)
CKH	R-time (min)	11.4 (IQR 9.3 to 14.2)
	K-time (min)	2.3 (IQR 1.8 to 3)
	Angle (°)	62 ± 7
	MA (mm)	54 (IQR 49 to 57)
CRT	R-time (min)	0.7 (IQR 0.5 to 0.8)
	K-time (min)	1.7 (IQR 1.3 to 2.1)
	Angle (°)	743(IQR 69 to 76)
	MA (mm)	57 (IQR 50 to 60)
	Ly 30 (%)	0.95 ± 0.95
	N = 33	
CFF	MA (mm)	19.8 ± 5.1

Abbreviations: PTT: Partial thromboplastin time; ACT: activated clotting time; UFH: unfractionated heparin; CK: kaolin activated intrinsic pathway assay; CKH: kaolin activated intrinsic pathway assay with additional heparinase; CRT: rapid TEG® assay; CFF: functional fibrinogen assay.

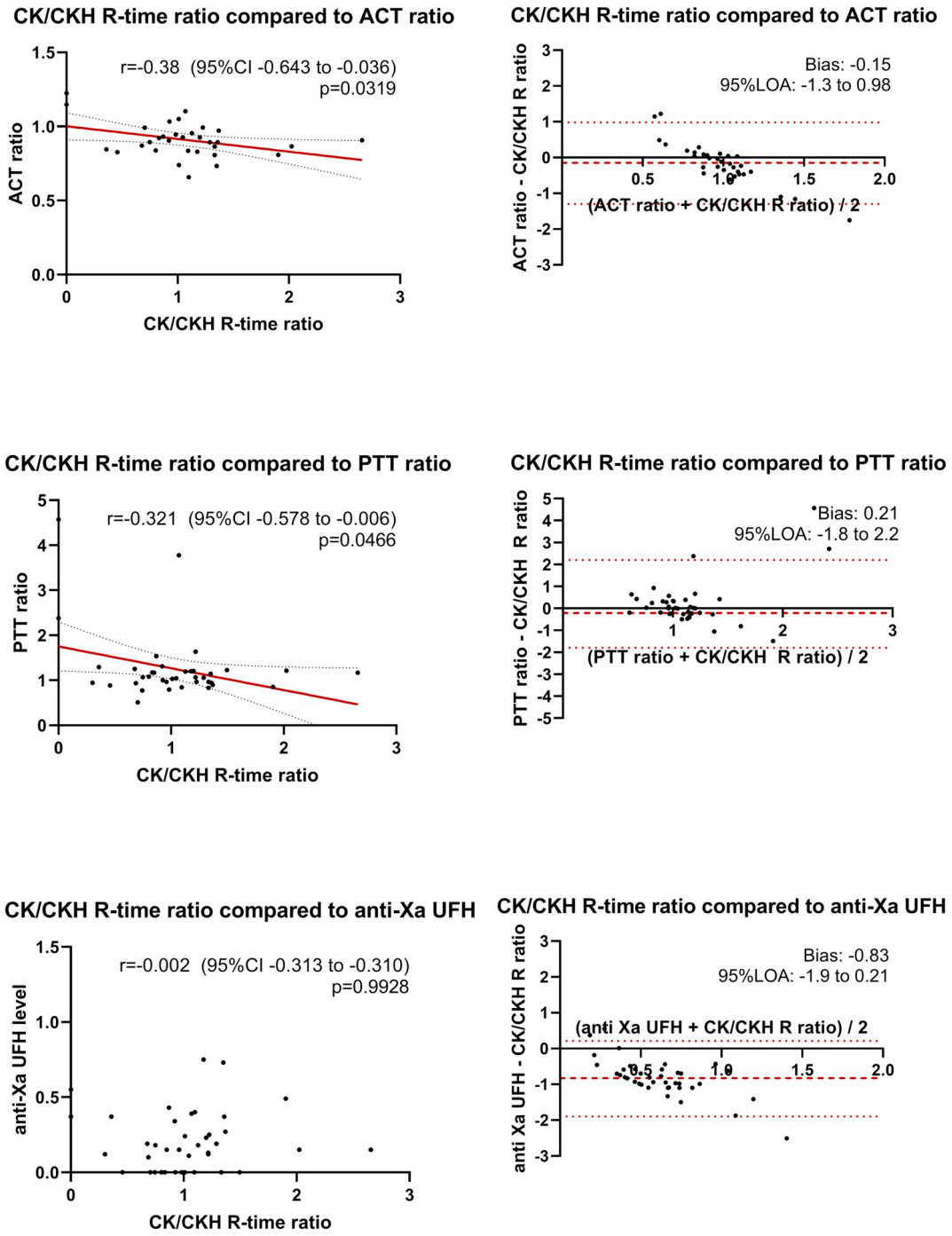

Fig. 2. Correlation analysis and Bland-Altman analysis of the thrombelastography parameter "CK/CKH R-time ratio" with respective conventional coagulation parameters "activated clotting time" (ACT) a), "partial thromboplastin time" b), and the "anti-Xa UFH level" c).

95%CI −0.251 to 0.43; $p = 0.5740$).

Left: The relationship of the respective parameters is indicated using the Pearson's correlation coefficient ("r"), indicated together with its 95%-confidence interval (95%CI) and the accompanying *p*-value. Right: Bland and Altman plots: mean bias of two respective measurements is given. The dashed red line represents the mean bias of the two ratios, the red dotted lines represent the limits of agreement (LOA).

3.2. TEG R-time dependent grouping in patients with and without successful heparin reversal

Based on the TEG-derived CK/CKH R-time ratio patients were divided in two groups. Group one consisted of 24/40 (60%) children with an CK/CKH R-time ratio below or equal 1 and group two consisted of 16/40 (40%) children with an CK/CKH R-time ratio above 1.

When comparing the dichotomized results of the two patient's group (with and without incomplete heparin reversal according to the CK/CKH R-time ratio) there was no significant correlation between the TEG-derived CK/CKH R-time ratio and the results of the PTT-ratio, the ACT-ratio or the anti-Xa UFH (Table 3).

4. Discussion

The reversal of heparin action is a very important aspect during

Table 3
Comparison of the different assays regarding their results to detect residual heparin in relation to the CK/CKH R-time ratio.

	CK/CKH R-time ratio ≤ 1 indicating complete reversal	CK/CKH R-time ratio > 1 indicating incomplete reversal	Fisher's exact test
ACT-ratio ≤ 1 indicating complete reversal	14	13	
ACT-ratio > 1 indicating incomplete reversal	5	0	0.0641
PTT ratio ≤ 1 indicating complete reversal	10	6	
PTT ratio > 1 indicating incomplete reversal	13	10	0.7521
Anti-Xa (UFH) ≤ 0.1 indicating complete reversal	10	3	
Anti-Xa (UFH) > 0.1 indicating incomplete reversal	14	13	0.1770

Abbreviations: PTT: Partial thromboplastin time; ACT: activated clotting time; UFH: unfractionated heparin.

coagulation management after CPB in congenital cardiac surgery. To the best of our knowledge, this study describes for the first time a novel viscoelastic resonance-based system (Haemonetics TEG® 6s) to detect incomplete heparin reversal in newborns, infants and small children undergoing congenital cardiac surgery. Due to the small blood volume of 400 μl needed for a complete viscoelastic analysis with this specific assay, this system might be beneficial for this special patient group. However, our study indicates that the available kaolin-activated assays (with and without heparinase) used in the new resonance-based TEG system reveal conflicting results when compared to other methods which aim to detect incomplete heparin.

Almost all congenital cardiac operations are performed using heparin as an anticoagulant. Heparin binds to AT III and leads to an almost 1000-fold acceleration of the inactivation of the clinically relevant factors IIa (thrombin) and Xa. To a lower extent heparin also inhibits factor IXa, XIa and XIIa. Protamine complexes with heparin and thereby inactivates the heparin effect. An important issue in the use of protamine is that its overdosing induces platelet dysfunction and can result in bleeding [5,6]. In current clinical settings heparin action and reversal is commonly monitored by bed-side measurements of the ACT. However, the ACT is best reliable with high circulating heparin levels and unfortunately shows poor reliability in newborns [7,8]. Distortion of ACT values can occur due to several individual factors not related to heparin. PoC viscoelastic tests allow for fast analysis of the clot initiation, amplification, strengthness and lysis in whole blood samples. To unmask the effects of circulating heparin, heparinase can be added to the assays to eliminate the anticoagulant effect of UFH. To a lower extent heparinase also eliminates the anticoagulant effect of low molecular weight heparins (LMWH). It has been shown that heparinase does not affect the baseline TEG measurements in the absence of circulating heparin [9]. A prolonged CK R-time suggests coagulation factor deficiency but if R-time decreases in the presence of heparinase (CKH R-time) incomplete heparin reversal is possible [3]. Thus, the R-time difference of CK minus CKH or the ratio of CK/CKH R-times are considered to be possible diagnostic tools for circulating heparin and are even affected by small amounts of heparin [3]. Laboratory data with the old rotational TEG system suggest that heparinase-based assays can detect increasing levels of circulation heparin [10]. This has been confirmed in clinical studies of children undergoing extracorporeal membrane oxygenation (ECMO) but a wide range of the results has been reported [11]. Therefore, the detection of incomplete heparin reversal after protamine administration remains challenging. In the clinical setting of CPB respective tests for heparin reversal are performed approx. 10 min after protamine administration, because heparin-protamine-complexes are cleared out of the plasma within this time. An optimal monitoring system for the detection of complete heparin reversal should be fast, available at the bedside, easy to perform and reliable. As described above ACT and PTT levels are influenced by several individual factors and do not solely measure heparin action. ACT measurements are known to be more reliable during phases of high circulating heparin levels. Thus, the ACT as well as the PTT are only surrogates of heparin activity. In contrast, Anti-Xa UFH level measurement is another possibility for the directly heparin activity. However, the laboratory analysis of the anti-Xa UFH levels as prolonged turn-around times of > 30 min from blood sampling to the final results. On the other hand, thrombelastography-derived R-times are known to be affected by circulating endogenous heparinoids (e.g. glycosaminoglycanes) and artificial colloids [12,13]. The latter were not used in the presented patient collective. However, glycosaminoglycanes (e.g. heparan sulphate, chondroitin sulphate and dermatan sulphate) that may become released from the endothelium and mast cells during CPB, may disturb TEG diagnostics because they can mimic a heparin like effect during the measurement [14]. This effect has previously been reported in adult patients during heparin-free ECMO support and anticoagulation with bivalirudin [15]. It is not entirely clear to which extent the data reported in our present study may have been affected by such issues. However, these facts indicate that the analysis of proper heparin reversal is challenging. Various assay-specific factors need to be taken into account when a suitable method for the perioperative analysis of heparin reversal is chosen. Of note, the comparison of conventional coagulation analyses and TEG results in our study may also have been influenced by the fact that there was a delay of 10–15 min between the initiation of TEG and conventional coagulation analyses. This time delay is caused by the transport time of the blood sample scheduled for conventional coagulation analysis. It has been reported that time frames up to 90–120 min in between sampling and analysis seem not to have a relevant impact on the results of coagulation measurements [16–18]. However, we cannot completely rule out a potential bias caused by the time delay in between the initiation of TEG and conventional coagulation assays.

4.1. Limitations

The study reported herein has several limitations. The presented data were retrospectively collected from clinical databases. Therefore, prospective validation is still missing and the transferability of our results to global clinical practice is limited. As described above some children needed to be excluded from data analysis because datasets were not completely retrievable. Also, TEG and conventional coagulation analyses were not initiated at the same time point because the blood sample for the conventional analyses had to transported to the central laboratory which caused the above-mentioned time delay of approximately 10 to 15 min.

4.2. Conclusion

Coagulation disorders in pediatric patients undergoing cardiac surgery with the use of CPB are a diagnostic and therapeutical challenge [7,19]. Incomplete heparin reversal is just one component of the CPB-associated coagulopathy besides many other factors including hypofibrinogenemia, platelet dysfunction and clotting factor deficiency. Ruling out residual heparin action is an important initial step in various

TEG-based algorithms in adults [20], yet algorithms for pediatric patients are missing up to date. As laboratory results e.g. PTT and anti-Xa levels are not readily available within a short period of time, intraoperative decisions still rely on ACT levels. However, ACT measurements are known to be inaccurate for the detection of low heparin levels. This situation may be improved by TEG. Our data show conflicting results with the use of different coagulation assays to identify incomplete heparin reversal. The new viscoelastic resonance-based TEG might help in the management of heparin reversal during CPB in pediatric cardiac surgery. However, further studies should be undertaken to evaluate, whether in comparison to other techniques, the R-time based management of incomplete heparin reversal by TEG might reduce blood loss and ultimately improve the outcome of children undergoing congenital cardiac surgery.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2019.08.008.

Declaration of competing interest

Harry Magunia and Andreas Straub have received speaker's honoraria from CSL Behring GmbH, Marburg, Germany. Andreas Straub has received speaker's honoraria from Aspen Germany GmbH, Munich, Germany. Sebastian Schenk, Christian Schlensak, Vanya Icheva, Peter Rosenberger and Martina Nowak-Machen have no conflicts of interest to report.

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Abbreviations

ACT: activated clotting time
Anti-Xa: anti-factor Xa
ATIII: antithrombin-III
CPB: cardio-pulmonary bypass
ECMO: extra corporeal membrane oxygenation
LMWH: low molecular weight heparins
POC: point of care
PTT: partial thromboplastin time
TEG: thrombelastography
UFH: unfractionated heparin