

Calculation of APTT and PT Reference Intervals from Patient Data and Evaluation of Test Utilisation in Surgical Patients

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Introduction

- ▶ Reference intervals (RI) are useful for providing information to clinicians for
 - ✓ **screening**
 - ✓ **decision- making**
 - ✓ **follow up disease**
- ▶ Results supported by RI → aid interpretation
- ▶ Clinicians compare the test results and RI to estimate patient's health status
- ▶ The concept of RI is well defined and is based on
 - fixed percentage of reference population within interval described by upper and lower reference limits (RLs)***



Generating the RIs is **responsibility** for all laboratories

use RI derived elsewhere (kit insert)*

determine their own interval for use with their population and analytical methods

- Recommended approach is having **own RI** for every laboratories.
- Population, ethnic, diet, laboratory, technique and selection of reference groups could be different.
- * Published RIs may not be compatible with existing analytical methods and tested populations

10/ RESULTADOS
El tiempo de trombotoplastia parcial activada de las muestras analizadas aparece en tiempo real en la pantalla electrónica por el operador, en la pantalla del aparato (ver el "Manual del Operador"). El resultado debe interpretarse en función del estado clínico y biológico del paciente.
Si el aparato señala que los resultados obtenidos para los controles se salen fuera del intervalo de valores indicados en la hoja incluida en el estuche, es preciso asegurarse del buen funcionamiento de todo el sistema: condiciones de ensayo, reactivos, plasmias en los que se efectuó el test, etc. Si es necesario, repite las muestras.

11/ LIMITACIONES
• En general el STA[®] - Cephascreen[®] es insensible a las deficiencias precoagulantes. Se ha mencionado en la literatura que los pacientes homogocigos con deficiencia precoagulante no manifiestan ningún episodio hemorrágico particular (8).
• Cuando se realice un control de terapia con heparina, cualquier liberación de factor plasmatina (aPTT) es así es un potente inhibidor de heparina, representará una importante fuente de error.
- Reciente la sangre en tubos plásticos, siliconizados, o CTAD.
- Realice la centrifugación dentro de 1 hora después de la toma de muestras si la sangre fue recolectada en el anticoagulante convencional (citrat) y dentro de 4 horas si la sangre fue recolectada con tubos CTAD.

12/ INTERVALO DE REFERENCIA
Los valores normales pueden variar de acuerdo a las condiciones locales (como tipo de población). Es necesario por lo tanto que cada laboratorio establezca sus propios rangos normales y los valores de control aceptables para su población (anal particular de pacientes). En general, los valores son considerados normales si se encuentran dentro del rango siguiente: media \pm 2 desviaciones estándar (2 \pm 2SD) (5).
Por ejemplo: se examinaron con el STA[®] - Cephascreen[®] 357 plasmias humanas normales utilizando el analizador STA[®]. El tiempo medio observado fue de 29.2 segundos, con una desviación estándar de 2.8 segundos. Se observó un APTT estadísticamente prolongado en recién nacido. En cambio, se observó tiempos más breves en la población de edad avanzada (4).

13/ CARACTERÍSTICAS DEL MÉTODO
Los resultados de los estudios de reproducibilidad intra e inter-serie obtenidos en STA[®] están indicados en las tablas siguientes:

	Reproducibilidad intra-serie		Reproducibilidad inter-serie	
Muestra	Muestra 1	Muestra 2	Muestra 3	Muestra 4
n	21	21	10	10
\bar{X} (s)	29.8	47.2	29.8	48.0
SD (s)	0.19	0.60	0.42	0.44
CV (%)	0.6	0.8	1.4	0.9

14/ VARIANTES
Los capítulos 1, 2, 3, 4, 5, 6 y 11 precedentes, son también válidos para la determinación con el reactivo automatizado.

14.1. Preparación y conservación del reactivo
Mantener el reactivo a temperatura ambiente (18-25 °C) durante 30 minutos antes de su uso. Agitar muy vigorosamente o con un agitador tipo varillas la vialidad estanca durante 3 a 5 segundos para obtener una solución homogénea (no añadir el STA[®] - Reductor, ni agua de dilución perfurada). Una vez homogenizado y abierto, el reactivo es estable 24 horas a 20 \pm 1 °C y 4 días a 2-8 °C, durante todo el día largo de su uso.

14.2. Reactivos y materiales auxiliares
• STA[®]-CaCl₂ 0.625 M (REF 00625)
• Coag Control (R) + (B) (REF 00621) o System Control (R) + (B) (REF 00617) control normal y anómalo.
• Instrumento similar al ST ar[®].
• Equipamiento habitual en los laboratorios de análisis clínicos.

14.3. Plasmias a analizar y controles
Las plasmias a testar y los controles se utilizan han de estar sin diluir.

14.4. Identificación
Comparar el APTT del paciente con el control del APTT de referencia utilizado en el laboratorio. Mantenga el reactivo STA[®] - Cephascreen[®] a temperatura ambiente (18-25 °C) antes de su uso. Siga las instrucciones del fabricante para la determinación del APTT.
Por ejemplo:
En una muestra precalentada a 37 °C:
• Diluir el plasma (de referencia, del paciente o de control) 1 vol.
• STA[®] - Cephascreen[®] 1 vol.
• Incubar a 37 °C durante 4 min.
• Añadir el reactivo, añadir el STA[®] - CaCl₂ 0.625 M precalentado a 37 °C 1 vol.
Añadir el tiempo de coagulación (segundos).

14.5. Resultados
Léase en cuenta el tiempo de coagulación (segundos) del plasma de referencia y el del plasma normal de referencia. El resultado debe interpretarse en función del estado clínico y biológico del paciente. Comparar los resultados de los controles de plasma con los intervalos indicados en la hoja incluida en el kit. Si el aparato señala que los resultados obtenidos para los controles se salen fuera del intervalo de valores indicados en la hoja incluida en el estuche, es preciso asegurarse del buen funcionamiento de todo el sistema: condiciones de ensayo, reactivos, plasmias en los que se efectuó el test, etc. Si es necesario, repite las muestras.

14.6. Intervalo de referencia
Por ejemplo, se analizaron 30 plasmias humanas normales con el instrumento ST ar[®]. El tiempo medio observado fue de 29.7 segundos con una desviación estándar de 2.5 segundos.

14.7. Características del método
Los resultados de los estudios de reproducibilidad intra e inter-serie obtenidos en ST ar[®] están indicados en las tablas siguientes:

	Reproducibilidad intra-serie		Reproducibilidad inter-serie	
Muestra	Muestra a	Muestra b	Muestra c	Muestra d
n	20	20	10	10
\bar{X} (s)	29.8	50.5	30.8	49.9
SD (s)	0.50	0.45	0.45	0.59
CV (%)	0.9	1.0	1.4	1.2

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Los cambios significativos son indicados por los tres puntos en el margen.

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Defining the RI

➤ Direct approach

- Recommended way
- Reference population selected and sample then analyzed for this purpose
- Randomly selected patients
- Outlier exclusions must be well defined
- Difficulty with the definition of health and the prevalence of subclinical case (selection bias)
- Time-consuming
- Expensive
- Patient convenience factors
- Small number of cases may not reflect biological diversity
- Clinical and Laboratory Standard Institute EP28-A3C

➤ Indirect approach

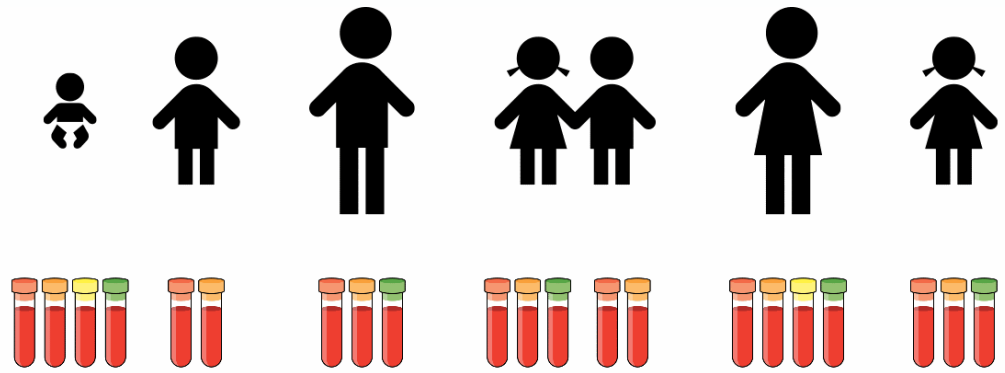
- Alternative way
- Results from specimen from routine purposes
- All results in database can be used and don't have to belong the reference population
- Patients come for screening, diagnosing or monitoring
- Faster
- Cheaper
- No patient inconvenience, discomfort
- No risk of ass. with generating new patient health info
- Large number of samples
- Same preanalytical and analytical process
- **Possible effects on diseased subpopulation (contamination)xx**
- **Influenced by extreme results**




EP28-A3c

Defining, Establishing, and Verifying
Reference Intervals in the Clinical Laboratory;
Approved Guideline—Third Edition

October 2010



 Ethical restrictions

Diagnostic and therapeutic decisions based on laboratory test results

Accurate reference intervals



Laboratory information systems

 Data-Mining

Systematic preoperative testing

- Routine testing is part of preoperative assessment designed to
 - 1) reduce the risks associated with the procedure and/or anaesthesia;
 - 2) detect unsuspected conditions
 - 3) provide a reference for postoperative assessment;
 - 4) evaluate the risk of postoperative complications with an independent predictive value



Preoperative testing is frequently overused against the recommendations of international guidelines

GUIDELINES

Pre-interventional haemostatic assessment

Guidelines from the French Society of Anaesthesia and Intensive Care

Fanny Bonhomme, Nadine Ajzenberg, Jean-François Schved, Serge Molliex, Charles-Marc Samama, for the French Anaesthetic and Intensive Care Committee on Evaluation of Routine Preoperative Testing

Recently the French Society of Anaesthesia and Intensive Care (*Société Française d'Anesthésie et de Réanimation [SFARI]*) issued recommendations for the prescription of routine preoperative testing before a surgical or non-surgical procedure, requiring any type of anaesthesia. Thirty clinical specialists performed a systematic analysis of the literature, and recommendations were then developed using the GRADE (*Grading of Recommendations Assessment, Development and Evaluation*) system. One part of these guidelines is dedicated to haemostatic assessment. The goal of pre-anaesthetic screening for congenital or acquired haemostatic disorders is to prevent perioperative haemorrhagic complications through appropriate medical and surgical management. Preoperative assessment of bleeding risk requires a detailed patient interview to determine any personal or family history of haemorrhagic diathesis, and a

physical examination is necessary in order to detect signs of coagulopathy. Laboratory investigation of haemostasis should be prescribed, not systematically, but depending on clinical evaluation and patient history. Standard tests (prothrombin time, activated partial thromboplastin time, platelet count) have a low positive predictive value for bleeding risk in the general population. Patients with no history of haemorrhagic diathesis and no conditions liable to interfere with haemostasis should not undergo pre-interventional haemostasis testing. Conversely, the existence of a positive history or a disease that could interfere with haemostasis should be an indication for clinically appropriate testing.

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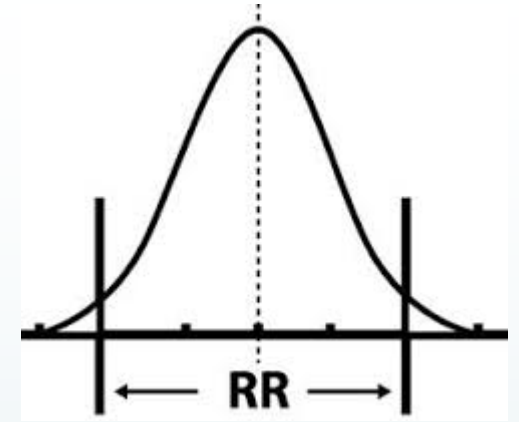


Aims

verify the reference intervals of our own laboratory by indirect procedure for activated partial thromboplastin time (APTT) and prothrombin time (PT)

investigate whether preoperative coagulation test requests are necessary.

Materials and Methods



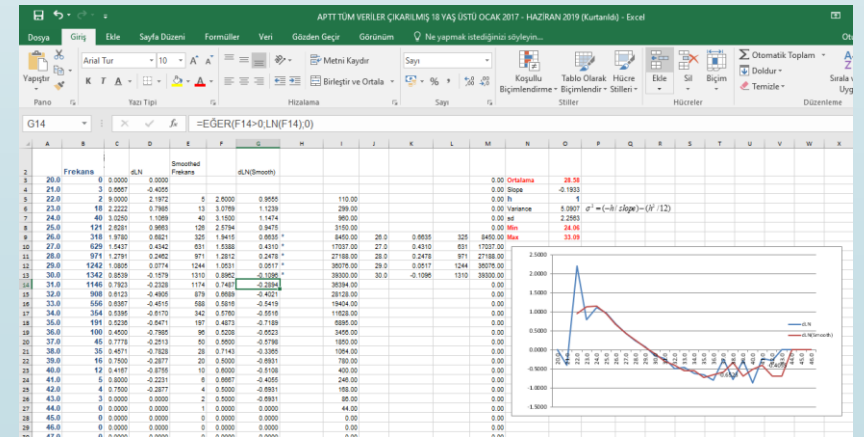
Subject data collection for RIs

- ▶ Subject data were selected retrospectively by screening coagulation results from hospital LIS between January 2017- June 2019
- ▶ We eliminated the test requests made by clinics of Emergency Department (Child and Adult), Anesthesia and Reanimation, Obstetrics and Gynecology, Nephrology, Infectious Disease, Pediatric and Adult Hematology outpatients, inpatients and intensive care units.
- ▶ Repeated test requests were eliminated.



- Subject data collection for preoperative coagulation testing
- Preoperative APTT and PT requests were used between July 2018 - June 2019 (for 1 year)
- Cardiology, Cardiovascular surgery and Oncosurgery patients and repeated test requests were eliminated.
- Laboratory analysis
- An evacuated tube system of 4 mL plastic tube containing %3.2 buffered trisodium citrate (Becton Dickenson Diagnostics, Franklin Lakes) was used
- All samples were centrifuged at 2500 g for 10 min
- APTT and PT levels were assayed on automated Stago Sta R Max coagulation analyzer.
- Kit insert RIs are APTT 29.2 ± 2.8 (23.6-34.8) sec } for all ages and sex
- PT 13.5 ± 1.8 (11.7-15.3) sec }

- Statistical methods
- We performed Excel macro with Bhattacharya procedure used for determination of RIs
- After RIs were calculated, preoperative patients' results and calculated RIs were compared.

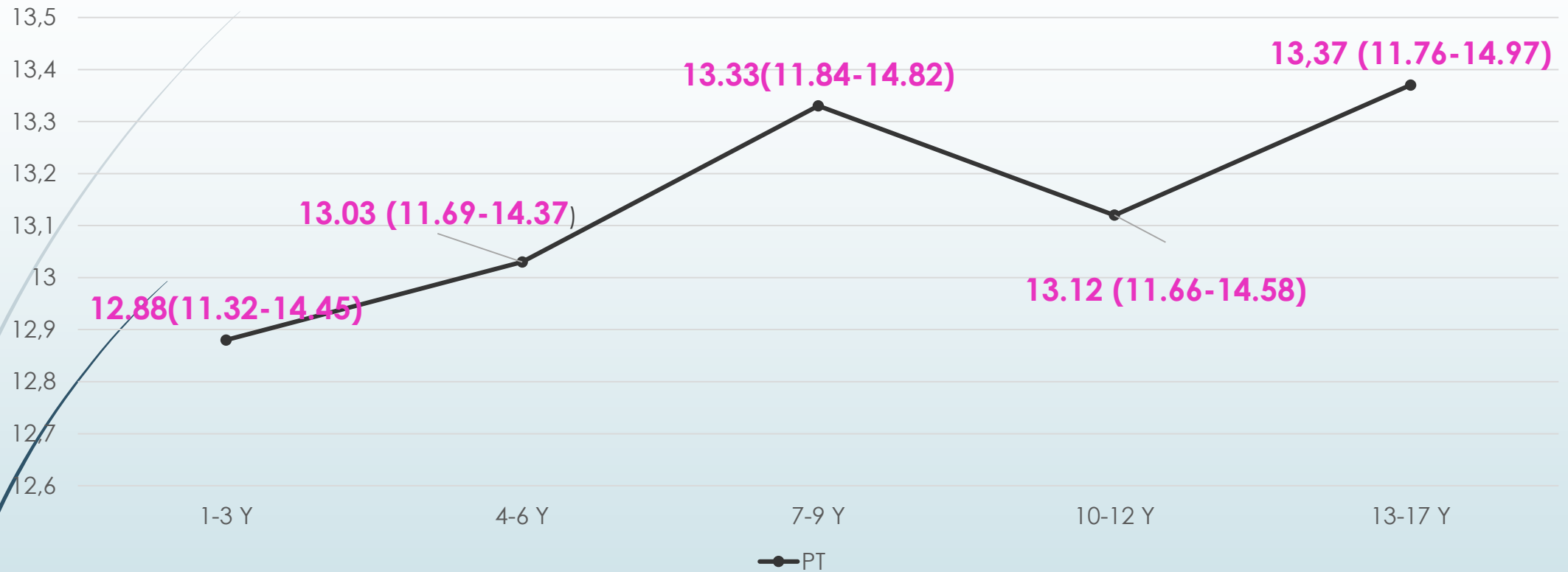


Results

Test parameters	APTT (sec) n=13611 (h=1)		PT (sec) n=13062 (h=0.5)	
	Mean	Lower - Upper limit	Mean	Lower - Upper limit
Age groups (years)				
1-3	29.97	24.88-35.05	12.88	11.32-14.45
4-6	30.39	25.70-35.07	13.03	11.69-14.37
7-9	29.71	25.94-33.48	13.33	11.84-14.82
10-12	30.49	25.91-35.07	13.12	11.66-14.58
13-17	30.47	26.14-34.79	13.37	11.76-14.97

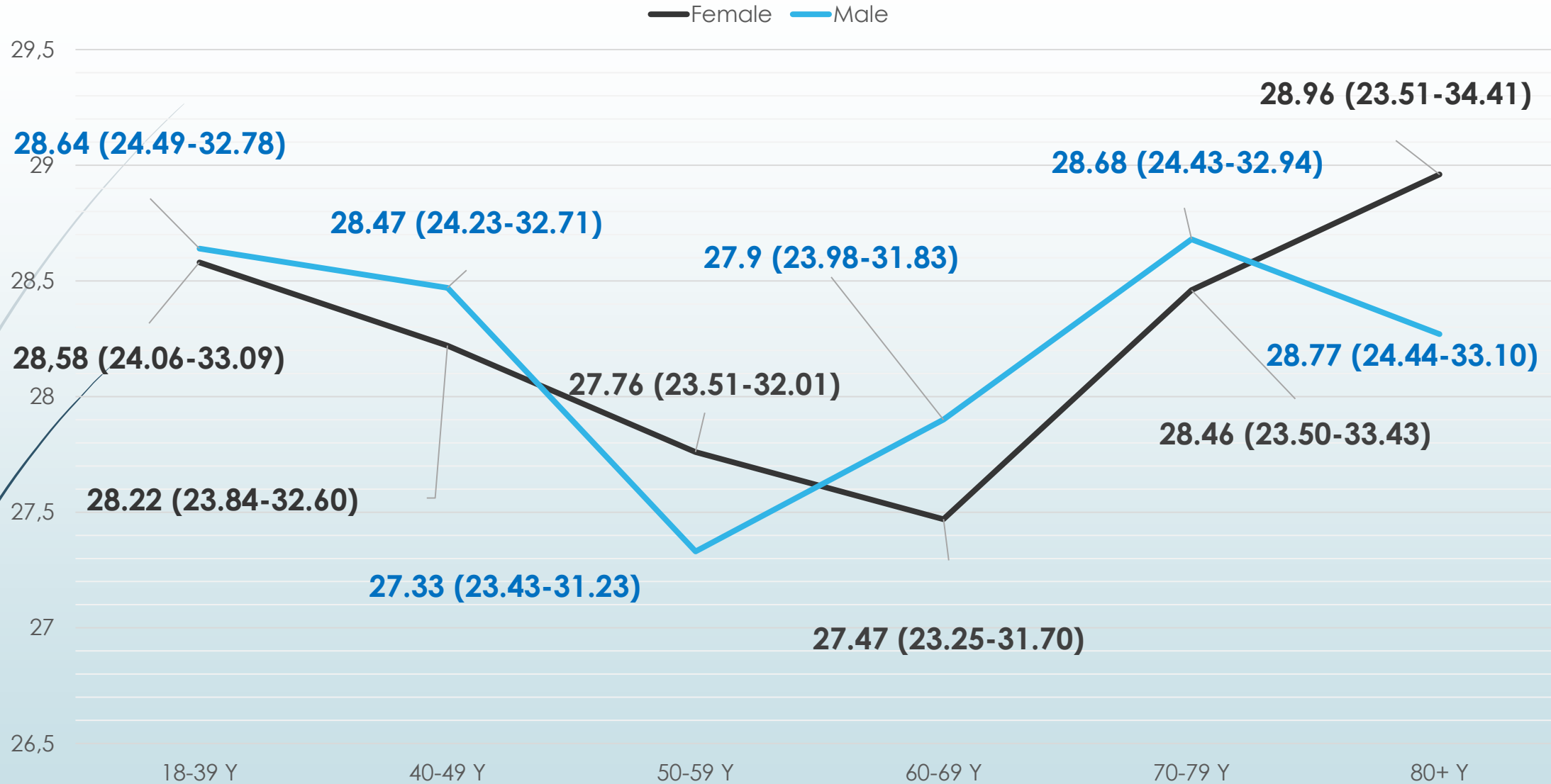
Table 1: Pediatric APTT and PT (sec) mean levels and reference limits in subgroups according to age.

Means of PT (sec) for children



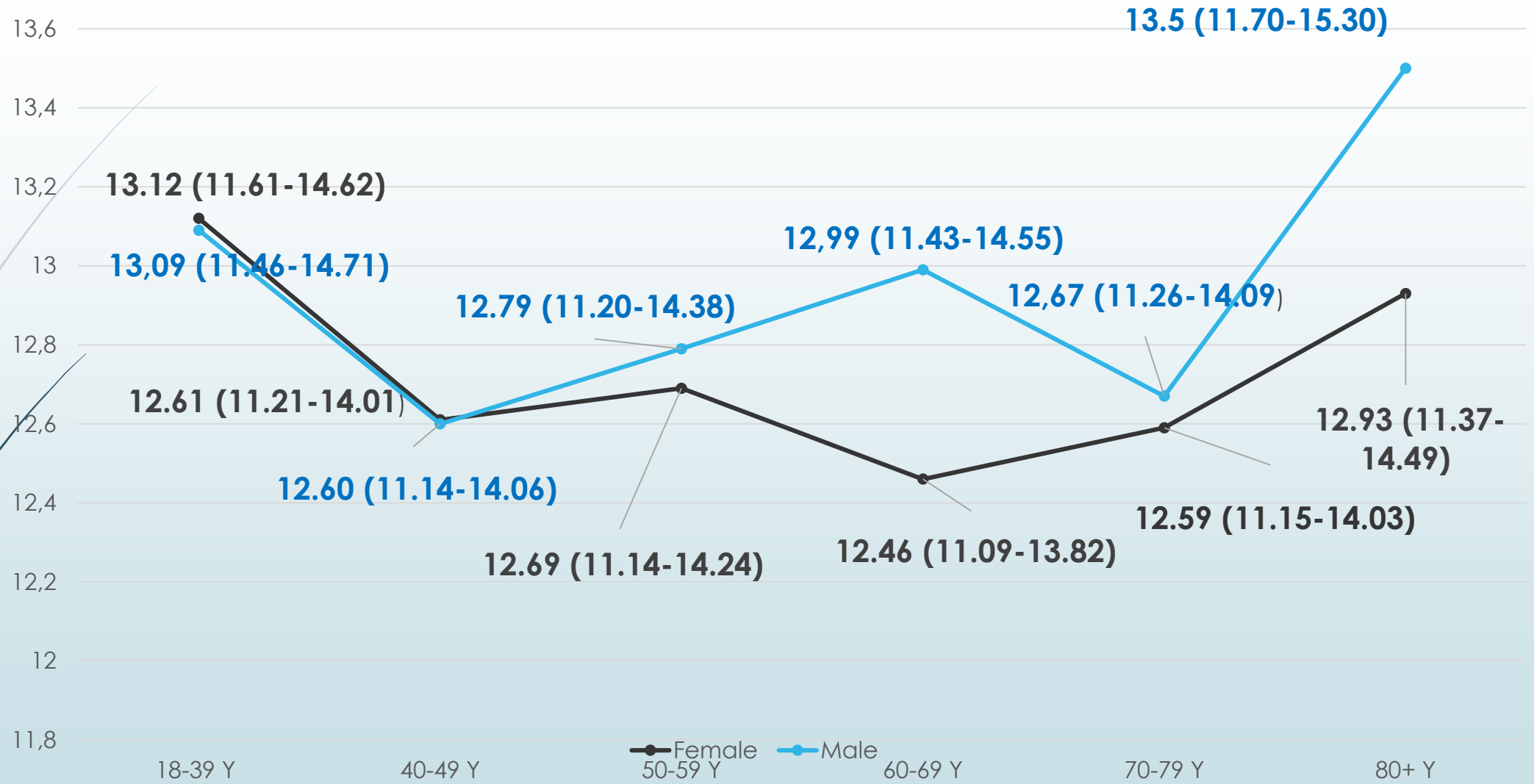
Graph 1: Means and reference limits of PT for children according to age

Means of APTT(sec) for adults



Graph 2: Means and reference limits of APTT for adults according to age and sex

Means of PT (sec) for adults



Graph 3: Means and reference limits of PT for adults according to age and sex

Ages (years)	Total n of operated patients	Test parameter (sec)	Below lower limit	Above upper limit	N of patients within the RI	% of patients within the RI	% of patients within the Present RI
1-3	114	APTT	6	7	101	87	89
		PT	0	34	80	70	85
4-6	114	APTT	8	7	99	87	94
		PT	0	34	80	70	90
7-9	121	APTT	3	16	102	84	91
		PT	0	33	89	73	84
10-12	82	APTT	5	6	71	87	91
		PT	0	33	49	60	78
13-17	156	APTT	4	11	141	90	94
		PT	1	56	99	64	72

Table 2 : Status of preoperative coagulation tests for children

Ages (years)	Sex	Total n of operated patients	Below lower limit	Above upper limit	N of patients within the RI	% of patients within the RI	% of patients within the Present RI
18-39	F	984	29	142	818	83	93
	M	887	34	146	707	80	91
40-49	F	298	4	49	245	82	93
	M	386	8	59	319	83	92
50-59	F	325	6	54	265	82	91
	M	343	2	87	254	74	92
60-69	F	343	2	87	254	74	92
	M	353	10	94	249	71	86
70-79	F	243	7	40	196	81	85
	M	217	3	58	156	72	85
80+	F	183	13	27	143	78	80
	M	131	4	25	102	78	78

Table 3: Status of preoperative APTT (sec) for adults

Ages (years)	Sex	Total n of operated patients	Below lower limit	Above upper limit	N of patients within the RI	% of patients within the RI	% of patients within the Present RI
18-39	F	984	8	187	789	80	88
	M	887	1	279	607	68	79
40-49	F	298	0	78	220	73	92
	M	386	0	127	259	67	87
50-59	F	325	0	56	269	83	90
	M	343	0	89	254	74	83
60-69	F	343	0	89	254	74	91
	M	353	0	91	262	74	84
70-79	F	243	0	105	138	57	83
	M	217	0	115	102	47	79
80+	F	183	0	87	96	52	74
	M	131	0	40	91	70	70

Table 4: Status of preoperative PT (sec) for adults



Discussion

- ▶ Laboratory specific RIs are important for accurate interpretation of patient results. Indirect methods could be a good solution for this purpose.
- ▶ Minimal elevation of PT may be the only clue for coagulopathy.
- ▶ APTT levels under the minimum reference limit is related to risk prediction for myocardial infarction and thromboembolic events.
- ▶ In accordance with the literature, perioperative events were not different in patients with or without preoperative testing.
- ▶ The French Society of Anaesthesiology and Intensive Care (SFAR) recommend that bleeding and family history should be asked before requesting the coagulation tests
- ▶ Recent studies continue to report over-requesting of preoperative tests reflecting the lack of clear guidelines or consensus.

Thank you..

