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Coagulation testing in patients using anticoagulants

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Anticoagulants such as low molecular weight heparin (LMWH), vitamin K antagonists (VKA), and the direct oral anticoagulants (DOACs) dabigatran and rivaroxaban are frequently used in clinical practice. Clinical laboratories analyse samples for different purposes, without knowledge of the presence of therapeutic anticoagulants, possibly leading to incorrect results. Moreover, as in emergency situations it is often unknown which anticoagulants have been used, the lack of specificity could be an issue in management of the patient. We therefore determined the influence of various anticoagulants on routine as well as dedicated coagulation tests.

Methods

Sample collection

Left-over citrated blood samples were obtained from patients being prescribed various anticoagulants (LMWH, unfractionated heparin (UFH), VKA and dabigatran) at the time of sampling. Samples were also collected from patients using combinations of the mentioned anticoagulants. The collection of these samples was performed daily using the data extraction tool Gaston (Medecs, Eindhoven, The Netherlands). Samples obtained from orthopaedic patients using rivaroxaban prophylactically after knee or hip replacement surgery, were kindly provided by the laboratory of Máxima Medical Center. The collection of blood samples was not timed with intake of medication. Only left-over plasma was used and venepunctures were performed as part of normal routine care. Each plasma sample was anonymized for the study. The study was conducted in accordance the local medical ethical protocol as prescribed by Dutch law.

The following groups were obtained: VKA (n=22), LMWH (n=30), VKA+LMWH (n=28), UFH (n=6), VKA+UFH (n=3), dabigatran (n=26) and rivaroxaban (n=10). The sample size of the VKA+UFH group (n=3) was considered too low and this group was therefore excluded from further analysis.

Coagulation assays

All analyses were performed on the STA-R Evolution Analyzer (Stago, Asnières sur Seine, France). The following routine assays were evaluated: PT (Neoplastin R, Stago, reference range 12.0-14.5 sec), aPTT (STA Cephascreen, Stago, reference range 30.0-41.0 sec) and thrombin time (TT, Thrombin Time, Stago, reference range 20.0-25.0 sec). The dedicated dabigatran assay was LD dTT (laboratory developed diluted thrombin time, for which Stago's thrombin reagent was used and 8 times sample dilution with normal plasma was applied). For rivaroxaban the anti-Xa assay (Liquid anti-Xa, Stago) was used. The anti-Xa assay was also used for LMWH, which was then calibrated with the Multi Hep calibrator (Stago). Method validation of these assays was described previously (1). The limits of detection (LOD) for LD dTT, anti-Xa rivaroxaban and anti-Xa heparin was 17.4 ng/mL, 24.0 ng/mL and 0.09 U/mL, respectively. These were determined by analysis of five blank samples in five-fold on two separate days. The LOD was calculated by the mean value in the blank plus two times the standard deviation.

Results

Median values and ranges established with the coagulation assays in the different patient groups are described in Table 1 and Figure 1.

In dabigatran samples with a concentration range of 15.1-438 ng/mL, the anti-Xa assay reported up to 17.8 ng/mL rivaroxaban (which was <LOD) and 0.11 U/mL LMWH. The INR remained below the therapeutic range for coumarins. All samples had a PT and APTT that was higher than the upper reference limit (URL; maximum values 1.62xURL and 2.05xURL, respectively). In all but three samples the TT was elevated to a level above the upper reporting range. One dabigatran sample had a TT within reference limits.

Rivaroxaban samples (16.6-110 ng/mL) reported presence of dabigatran up to 24.2 ng/mL when analysed with the dTT assay. When calibrating the assay for LMWH, the samples reported up to 2.54 U/mL LMWH. The INR remained below the therapeutic range for coumarins. The APTT and PT were elevated in all rivaroxaban samples (maximum values 1.61xURL and 1.43xURL, respectively). The TT was elevated marginally in 20% of all samples.

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Table 1. Results of various coagulation assays (dTT dabigatran, anti-Xa heparin or rivaroxaban, PT, INR, APPT and thrombin time) performed in patients using different anticoagulants.

	N	dTT [ng/mL]	Anti-Xa (Rivaroxaban) [ng/mL]	Anti-Xa (LMWH) [U/mL]	INR [-]	PT [sec]	APTT [sec]	TT [sec]
Dabigatran	26	167 (15.1 – 438)	11.8 (0.00 – 17.8)	0.07 (0.00 – 0.11)	1.5 (1.2 – 1.8)	19.8 (15.3 – 23.5)	62.1 (41.8 – 84.0)	>120 (21.7 – >120)
Rivaroxaban	10	19.2 (16.7 – 24.2)	50.3 (16.6 – 110)	1.15 (0.11 – 2.54)	1.5 (1.3 – 1.7)	19.7 (16.7 – 23.3)	48.4 (42.6 – 58.7)	21.4 (19.7 – 22.7)
LMWH	30	25.8 (15.9 – 36.5)	36.2 (6.57 – 53.2)	0.53 (0.05 – 1.40)	1.5 (1.1 – 2.1)	19.9 (14.3 – 28.6)	85.1 (35.8 – 138)	>120 (21.4 – >120)
VKA	22	20.0 (0.00 – 27.5)	11.2 (3.26 – 16.4)	0.05 (0.00 – 0.08)	3.3 (1.5 – 7.8)	46.6 (20.3 – 117)	64.2 (43.0 – 105)	21.1 (17.9 – 27.0)
VKA&LMWH	28	21.7 (0.00 – 30.8)	18.4 (14.1 – 27.9)	0.23 (0.03 – 0.85)	3.6 (1.9 – 7.7)	50.5 (25.2 – 116)	79.2 (49.6 – 174)	27.0 (15.1 – >120)
UFH	6	31.6 (19.2 – 135)	1.1 (0.58 – 1.8)	63.4 (25.8 – 138)	1.5 (1.3 – 1.8)	19.2 (17.2 – 23.5)	>240 (74,0 – >240)	>120 (>120)

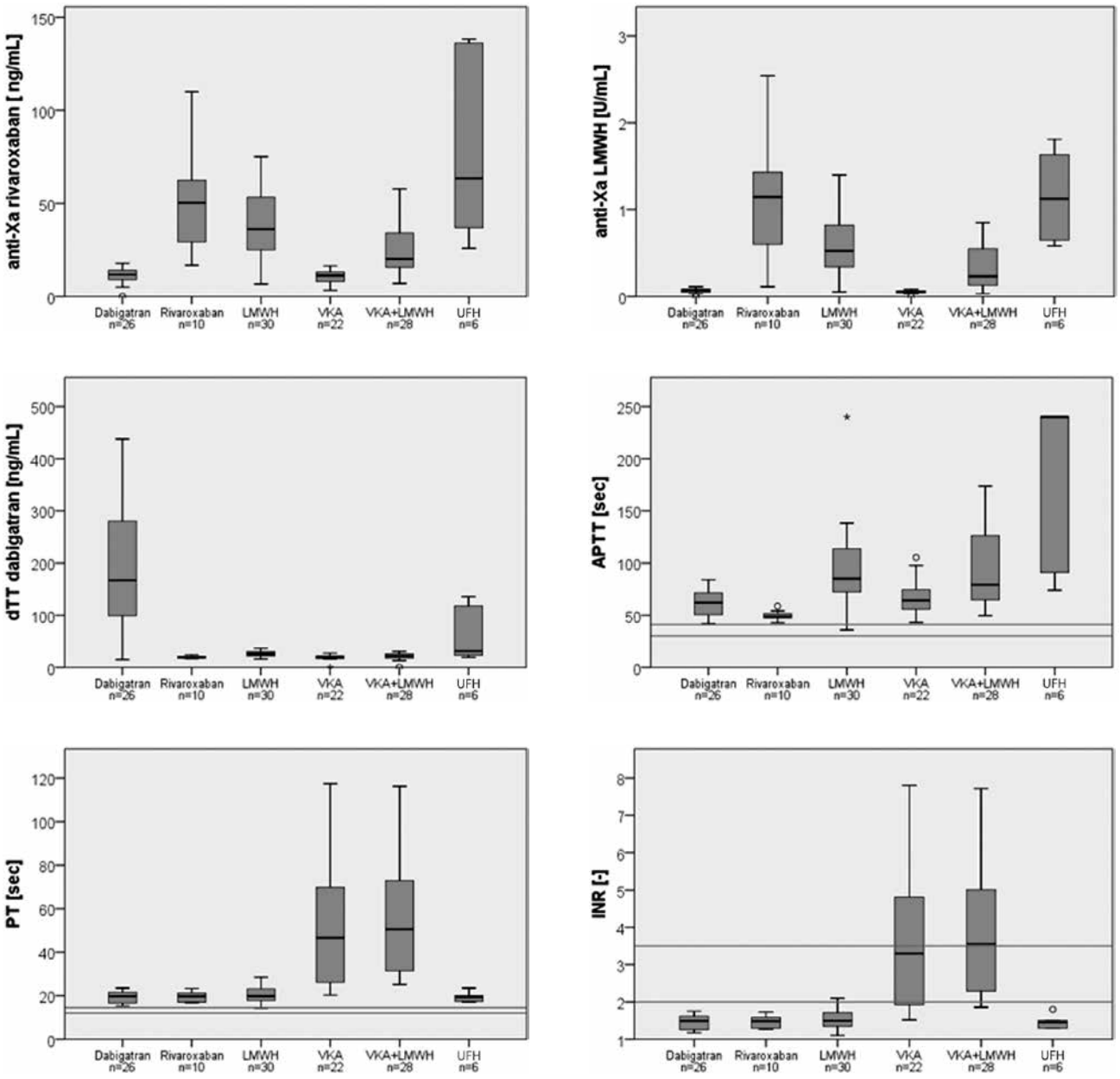

Figure 1. Results of various coagulation assays performed in patients using different anticoagulants. [A] anti-Xa rivaroxaban, [B] anti-Xa LMWH, [C] dTT dabigatran, [D] APTT, [E] PT, [F] INR. The upper and lower reference limits for the PT and APTT as well as the therapeutic limits for the INR are indicated as horizontal lines.

Although the LMWH samples' concentration did not cover the full therapeutic range of 0.6-2.0 U/mL using the anti-Xa assay with UFH calibration, a dabigatran concentration of up to 36.5 ng/mL and a rivaroxaban concentration of up to 53.2 ng/mL was measured. The INR again remained below the therapeutic range for coumarins. The APTT was elevated in all samples (maximum value 3.37xURL) and the PT was only within reference range in one sample (maximum value 1.97xURL). The TT varied greatly in the LMWH-samples, with 13% of the samples within the reference range and 70% above the upper reporting range.
In the samples obtained from VKA-using patients (INR range 1.52-7.8) a dabigatran concentration of up to 27.5 ng/mL was established. No relevant anti-Xa activity could be detected in these samples (rivaroxaban 16.4 ng/mL and LMWH up to 0.08 U/mL, which were <LOD). The APTT was elevated in all samples (maximum value 2.56xURL). The TT was marginally elevated in 41% of the samples.

Discussion

In our study, the dedicated coagulation tests dTT and anti-Xa showed limited interference by oral anticoagulants. This can however still be of relevance in clinical practice, when information of medication use by the patient is lacking. As in the near future specific antidotes for dabigatran and rivaroxaban will become available, conclusions made on drug identity in an unknown patient should be errorless.
As LMWH exhibits anti-Xa as well as some anti-IIa activity it can be expected that dedicated DOAC-monitoring assays (dTT and anti-Xa) are more or less affected by use of different LMWH. This was confirmed by a high extent of interference of LMWH on the rivaroxaban assay and a relatively low interference on the dTT assay. UFH interfered significantly on all tests. In clinical practice however, patients treated intravenously with unfractionated UFH are adequately identified.
Although elevated in all samples, our APTT and PT reagents were relatively insensitive for dabigatran and rivaroxaban. However, these reagents were unexpectedly sensitive for LMWH. In contrast to our findings, LMWH is mostly not considered to be of much influence on the APTT (2). It was unknown if the samples were obtained from patients on a therapeutic or prophylactic LMWH-regimen. However, samples with anti-Xa LMWH <0.6 u/mL had an APTT up to 138 sec (data not shown). It can therefore be expected that samples taken from hospitalized patients on prophylactic LMWH-therapy mostly have a relevantly elevated APTT, using our reagent.
The undiluted thrombin time is considered as a sensitive screening aid for dabigatran (3). This was confirmed in our study. The one sample in a patient with a normal thrombin time, had a low concentration of dabigatran (determined as 21.7 ng/mL). The thrombin time was however also sensitive for LMWH and showed marginal elevation in rivaroxaban and VKA samples. Therefore, care should be taken using this test as a screening test in an emergency aid setting.

Conclusions

The interference of the dTT and anti-Xa assays by other oral anticoagulants was limited, but could still be of clinical relevance. Use of adequate lowest reporting limits should be considered for quantification of direct oral anticoagulants when coagulation assays are used. According to our data, these limits are 27.5 ng/mL for dabigatran (maximum interference of rivaroxaban or VKA on the dTT) and 17.8 ng/mL for rivaroxaban (LOD of anti-Xa, which is higher than the maximum interference of dabigatran or VKA on the anti-Xa). The STA-APTT reagent showed high sensitivity to LMWH and VKA. In laboratories using this reagent, care should be taken when interpreting the APTT, especially in hospitalized patients.