Introduction

The prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin complex (TAT) are direct markers of in vivo thrombin generation. While F1+2 represents an activation peptide split off during the conversion of prothrombin to active thrombin, TAT is the result of thrombin inhibition by complex formation with antithrombin.

The active enzyme thrombin itself cannot be directly quantified due to its very short half-life time of less than 1 minute and rapid inactivation. A further, indirect marker of thrombin activity (and plasmin activity) is D-dimer, which represents a breakdown product of fibrin and is generated only when thrombin has converted fibrinogen to fibrin and thrombin-induced activated FXIII has cross-linked the fibrin. Plasmin activation always occurs concomitantly with fibrin formation.

The three thrombin generation markers—F1+2, TAT, and D-dimer—differ substantially with regard to their molecular mass, half-life time ($t_{1/2}$), and molar basal concentration in the peripheral blood.

With a half-life time of about 16 hours and its increase being relative to the amount of fibrin generated, D-dimer serves as a marker of longer-term thrombin generation, whereas F1+2 and TAT reflect short-term thrombin activity.

While current assays for TAT allow only quantification of hypercoagulability (increased TAT levels), the test systems for F1+2 allow detection of hyper- and hypocoagulability (increased as well as decreased levels). Furthermore, F1+2 is less affected by pre-analytical sampling artifacts due to its higher molar basal concentration.

Figure 1. Coagulation markers with their half-lives ($t_{1/2}$) and molecular weight (kD).[^1]
Prothrombin Fragment 1+2 (F1+2): A Sensitive Marker for In Vivo Thrombin Generation Activity

F1+2 is a sensitive marker of thrombin generation, reflecting the hypercoagulable state associated with a wide variety of conditions/diseases, e.g., old age, pregnancy, cancer, and cardiovascular disease. Its increase with age is related to the less-smooth and more-procoagulant surface of vessel walls with aging, increasing prevalence of hypertension, disturbed blood flow (stenosis), and decreased renal clearance.

Hypercoagulability, reflected by elevated F1+2 levels, can be induced by estrogens; application of estrogens for contraception or hormone replacement induces a slight increase in coagulation activation. The pronounced hormone increase during pregnancy accompanies a persistent pronounced hypercoagulability, with F1+2, TAT, and D-dimer increasing progressively during pregnancy.

F1+2 in uncomplicated pregnancy

<table>
<thead>
<tr>
<th>Trimester</th>
<th>F1+2 (pM)</th>
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<tbody>
<tr>
<td>1st trimester</td>
<td>206 ±50</td>
</tr>
<tr>
<td>2nd trimester</td>
<td>422 ±146</td>
</tr>
<tr>
<td>3rd trimester</td>
<td>576 ±162</td>
</tr>
<tr>
<td>Controls*</td>
<td>115 (69–229)</td>
</tr>
</tbody>
</table>

*Median, 2.5–97.5% range.

Determinants of F1+2 levels

- Increase with aging is associated with:
  - Decrease of clearance by renal filtration
  - Increased prevalence of vascular disease with more-procoagulant vessel walls
  - Increased prevalence of hypertension
  - Increased thrombogenicity of vessel walls
  - Disturbed blood flow (e.g., stenosis)

- Pregnancy
- Cancer

Increase of F1+2 with aging
F1+2 in Cardiovascular and Thromboembolic Disease

Elevated F1+2 levels are observed with:

- Stable and unstable cardiovascular disease (CVD)
- Acute coronary syndrome (ACS) and myocardial infarction (AMI)
- Stroke
- Atrial fibrillation (AF)
- Venous thromboembolism (VTE)

Several cardiovascular risk factors, such as smoking, hypertension, hypercholesterinemia, carotid intima-media thickness, and presence of heart failure, are associated with increasing F1+2 levels.4

F1+2: risk marker for recurrent events in cardiovascular disease

Increased F1+2 levels have been shown to be a prognostic marker for:

- Reinfarction in AMI patients5
- Mortality in patients with unstable angina6 and noncardioembolic stroke7
- Thromboembolic complications in cancer patients8
- Recurrent VTE after termination of anticoagulant therapy, additive to D-dimer9

### Hypercoagulability and risk for recurrent ACS in patients with a first AMI9

<table>
<thead>
<tr>
<th></th>
<th>With Recurrent Events</th>
<th>Without Recurrence</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1+2 (pM)</td>
<td>314 (207–666)</td>
<td>213 (170–299)</td>
<td>0.03</td>
</tr>
<tr>
<td>D-dimer (µg/L)</td>
<td>625 (382–1325)</td>
<td>360 (260–680)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Higher activation of coagulation (F1+2, D-dimer) on admission in patients with a first AMI was associated with a significantly increased risk for recurrent cardiovascular events.

F1+2 and mortality in stroke patients7

Patients in the acute or chronic phase of noncardioembolic stroke in general showed elevated F1+2 levels. Those with more pronounced hypercoagulability indicated by F1+2 above the median of 2.2 nM had an increased mortality.
**F1+2 and Venous Thromboembolic Events (VTE)**

Anticoagulation therapy in patients with VTE induces a suppression of coagulation activity and decrease of F1+2 levels. After discontinuation of anticoagulation, a re-increase of coagulation activity and F1+2 levels is seen, and higher levels of activation are associated with an increased risk for recurrent events.9

The association of malignant diseases with a high risk for thromboembolic disease has been recognized for more than 100 years. Coagulation activation markers such as F1+2, TAT, and D-dimer now allow quantification of the extent of coagulation activation and risk stratification with regard to manifestation of VTE.

**Risk predictors for VTE recurrence**

295 consecutive patients with a first episode of VTE were tested 1 month after stopping anticoagulation and followed up for a median time of 25 months.9

<table>
<thead>
<tr>
<th>Odds Ratio (95% CI)</th>
<th>p</th>
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<tbody>
<tr>
<td>Male sex</td>
<td>2.3 (1.1–4.8)</td>
</tr>
<tr>
<td>Elevated D-dimer</td>
<td>3.2 (1.5–6.6)</td>
</tr>
<tr>
<td>Elevated F1+2</td>
<td>2.6 (1.2–5.6)</td>
</tr>
<tr>
<td>Presence of residual venous occlusion</td>
<td>1.1 (0.5–2.4)</td>
</tr>
<tr>
<td>Common thrombophilia</td>
<td>0.6 (0.2–1.6)</td>
</tr>
<tr>
<td>Elevated D-dimer and F1+2</td>
<td>4.0 (1.6–10.3)</td>
</tr>
</tbody>
</table>

**F1+2 and D-dimer predict VTE in cancer patients**

The graph shows risk estimates for development of VTE in patients with new or progressing cancer (not under therapy at inclusion) according to:
1. Elevated D-dimer and F1+2 (15%)
2. Elevated F1+2 alone
3. Elevated D-dimer alone
4. Nonelevated D-dimer and F1+2 (5%)

(1 versus 2–4: Log rank test p < 0.001)

**Response of F1+2 to Anticoagulation Therapy**

The reduced activation of the procoagulant enzyme cascade in response to anticoagulant therapy is sensitively reflected by the decrease of F1+2 levels compared to nonanticoagulated controls.

**Decrease of F1+2 with increasing intensity of VKA therapy**

In the 1990s, the first studies investigating the intensity of anticoagulant therapy with vitamin K antagonist (VKA) demonstrated a relationship between intensity of VKA therapy (as indicated by INR) and the decrease of F1+2 levels. Median levels of F1+2 decrease with increasing intensity of VKA therapy. However, in certain patients F1+2 levels are considerably higher than expected, indicating a possibly insufficient suppression of coagulation activation.10

From the relationship between F1+2 levels and INR, Bruhn et al. postulated an optimized therapeutic target range of 0.4 to 1.2 for the ratio of F1+2 (in nM) to PT in INR, with ratios higher than 1.2 indicating suboptimal anticoagulation in a persistent hypercoagulable, thrombophilic state, and ratios below 0.4 indicating an oversuppression of coagulability and a potential bleeding risk.10,11

**Concept for therapy optimization:**

- Target F1+2/INR ratio of 0.4–1.210,11
**F1+2 and direct oral anticoagulants**

The suppression of coagulation activity by the new direct oral anticoagulants (DOACs) is reflected in a decrease of F1+2 levels in response to the thrombin inhibitor dabigatran, as well as the anti-Xa drugs rivaroxaban, apixaban, and edoxaban, compared to non-anticoagulated controls. However, when compared to VKA therapy, the effect of F1+2 suppression is less pronounced and does not exceed the lower limit of the reference range. The stronger hypocoagulable effect of VKA, with most patients showing F1+2 levels below the reference range, may be an indicator of the higher bleeding risk associated with VKA therapy. After discontinuation of DOAC therapy, irrespective of the drug previously applied, F1+2 levels increase back to high-normal to slightly elevated levels within 4 weeks.12-17

**Effect of rivaroxaban and warfarin on F1+2 in Japanese patients with atrial fibrillation**

F1+2 levels are not influenced by the CHADS2 score, but warfarin inhibits thrombin generation more aggressively than rivaroxaban.12

For thromboprophylaxis in patients undergoing orthopedic surgery, edoxaban has been shown to suppress coagulation activation more efficiently, as indicated by lower F1+2 and D-dimer levels compared to patients receiving LMWH prophylaxis.17

**Reduction of F1+2 by LMWH prophylaxis in thrombophilic pregnant women18**

(21 thrombophilic pregnancies with nadroparin prophylaxis, 20 pregnant controls)

In patients with hereditary thrombophilia such as FV Leiden, antithrombin, or protein C or S deficiency, slightly elevated F1+2 levels have been described. Furthermore, in pregnancy, another thrombophilic condition, F1+2 levels increase with gestation age. Presence of hereditary thrombophilia is an established risk factor for pregnancy complications such as thromboembolic events or miscarriage. The effect of a prophylactic LMWH therapy in pregnant women with thrombophilia (FV Leiden, lupus anticoagulants, protein C or S deficiency) is reflected by a significant reduction of in vivo thrombin generation over the course of pregnancy.
Influence of Different Oral Anticoagulants for VTE Therapy and Prophylaxis on F1+2 and D-dimer

In order to assess the efficacy and impact of different oral anticoagulants, a total of 130 samples were investigated at Kantonsspital Aarau (Switzerland) from hospitalized patients under MARCUMAR (VKA therapy) with different INR ranges (<2.0, 2.0–3.0, >3.0–4.5, >4.5) and compared with 10 patients on 20 mg rivaroxaban (VTE therapy) and 11 patients on 10 mg rivaroxaban (VTE prophylaxis) after knee endoprothesis (TEP) surgery. Three blood samples were collected from patients under rivaroxaban: before, 3 hours, and 6–8 hours after drug intake. F1+2 and high-sensitivity (hs) D-dimers were measured using the INNOVANCE® LOCI® F 1+2 † and LOCI High Sensitive D-Dimer (hs D-Dimer) ‡ Assays on the Atellica® COAG 360 System † (Siemens Healthineers, Marburg, Germany).

In patients on VKA therapy, F1+2 and hs D-dimers significantly decreased with increasing INR up to 3.0 (p < 0.05).

More intense VKA anticoagulation did not result in further decrease of coagulation activation markers.

†Not available for sale in the U.S.
‡Research use only.
Median F1+2 levels measured at different time points after drug intake showed more variation for the prophylactic rivaroxaban dosage than the higher, therapeutic dosage. At drug trough levels, F1+2 showed a more pronounced elevation compared to the levels measured around peak level (3 hours) or 6–8 hours after drug intake. With the therapeutic rivaroxaban dosage, a similar but much less-pronounced kinetic for F1+2 was observed.

However, both F1+2 and hs D-dimer were significantly lower in patients receiving the more-intense therapy regimen. On the other hand, the hypernormal F1+2 levels seen in the patients under prophylaxis may be the result of severe hypercoagulation induced by the surgical TEP procedure.

**F1+2, hs D-dimer, and rivaroxaban levels at different time points**

![Graph showing F1+2 and hs D-dimer levels over time for rivaroxaban 10 mg and 20 mg]

**Influence of rivaroxaban and VKA therapy on F1+2 and hs D-dimer**

Rivaroxaban therapy and prophylaxis seem to suppress the activation of coagulation in a dose-dependent way.

When comparing F1+2 and hs D-dimer for the three therapy regimens at trough levels, VKA therapy most efficiently suppressed thrombin generation reflected by F1+2.

However, fibrin formation reflected by D-dimer was significantly more suppressed under therapeutic rivaroxaban.

In order to confirm these first observations, the study will continue with enrollment of more patients.
Measurement of F1+2

The first routine immunoassay for determination of F1+2, the Enzygnost® F1+2 tube-based ELISA assay, was launched in 1990. The assay has been continuously improved by conversion to a microtiter plate format in 1995 (Enzygnost F1+2 micro Assay), a change from use of polyclonal to monoclonal antibodies in 2014 (Enzygnost F1+2 monoclonal Assay), and in 2017 by application of LOCI technology.

The INNOVANCE LOCI F 1+2 Assay† for the first time enables the application of this biomarker on a clinical routine coagulation analyzer, providing fully automated processing and single-sample STAT testing with a short turnaround time of 13 minutes, and allows F1+2 testing in emergency cases, day and night. In addition to less hands-on time, less calibration, and a larger assay measuring range compared to the previous ELISA assay, the precision has improved to CVs of 0.5–2%.

The INNOVANCE LOCI F 1+2 Assay† has been developed for fully automated use on the Atellica COAG 360 System.† All reagent components are stored onboard the analyzer, accelerating overall processing and allowing single-sample STAT analysis. LOCI technology is a highly sensitive, homogeneous immunoassay technology that eliminates in-between washing and separation steps. As only a very small sample volume is required, LOCI assays are less prone to interference effects and well-suited for pediatric application. Furthermore, LOCI technology provides high sensitivity with excellent precision over a large, dynamic measuring range.

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<table>
<thead>
<tr>
<th>F1+2 Assays</th>
<th>Reference Range</th>
<th>Measuring Range</th>
<th>Precision (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzygnost F1+2, Enzygnost F1+2 micro</td>
<td>0.4–1.1 nmol/L†</td>
<td>0.04–10.0 nmol/L</td>
<td>6–13</td>
</tr>
<tr>
<td>ELISA using polyclonal antibodies</td>
<td>(median 0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzygnost F1+2 monoclonal</td>
<td>69–229 pmol/L†</td>
<td>20–1200 pmol/L</td>
<td>6.8–11.8</td>
</tr>
<tr>
<td>ELISA using monoclonal antibodies</td>
<td>(median 115)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INNOVANCE LOCI F 1+2†</td>
<td>63.3–307.1 pmol/L**</td>
<td>15–5000 µmol/L</td>
<td>1.1–2.0</td>
</tr>
<tr>
<td>LOCI assay using monoclonal antibodies</td>
<td>(median 124.2)</td>
<td>(LoQ: 11.4)</td>
<td></td>
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</table>

†Not available for sale in the U.S.
§5th to 95th percentile.
**2.5th to 97.5th percentile.
Summary

F1+2 is a sensitive marker of in vivo thrombin generation, with elevated F1+2 levels indicating increased coagulation activation, e.g., in thrombotic states, consumption coagulopathy, or cancer.

Decreased or low-normal F1+2 levels are seen under anticoagulant therapy and may indicate a higher risk of bleeding.

F1+2 allows assessment of the activity of the coagulation system in response to different medications, e.g., in VTE, coronary syndrome, sepsis, etc.

F1+2 provides additive prognostic information that improves risk prediction for future cardiovascular or thromboembolic complications.

The INNOVANCE LOCI F 1+2 Assay† allows fully automated, single-sample STAT analysis of F1+2, with results available within 13 minutes, around the clock.

INNOVANCE LOCI F 1+2 Assay:†
The Fully Automated, Routine Assay for In Vivo Thrombin Generation

- Based on LOCI technology
- Runs on the Atellica COAG 360 routine coagulation analyzer
- Allows you to run single samples STAT

Potential Clinical Applications for F1+2

- Anticoagulation monitoring (traditional and new anticoagulants)
- Marker for non-overt DIC marker in ISTH guidelines
- Monitoring of pregnant women with thrombophilia/preeclampsia/HELLP syndrome
- Risk stratification for stroke in atrial fibrillation patients (additive to D-dimer)
- Risk stratification for thrombosis in cancer patients (together with D-dimer)
- Prognostic marker in patients with acute cardiac events in combination with troponin and NT-proBNP

†Not available for sale in the U.S.
References:
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