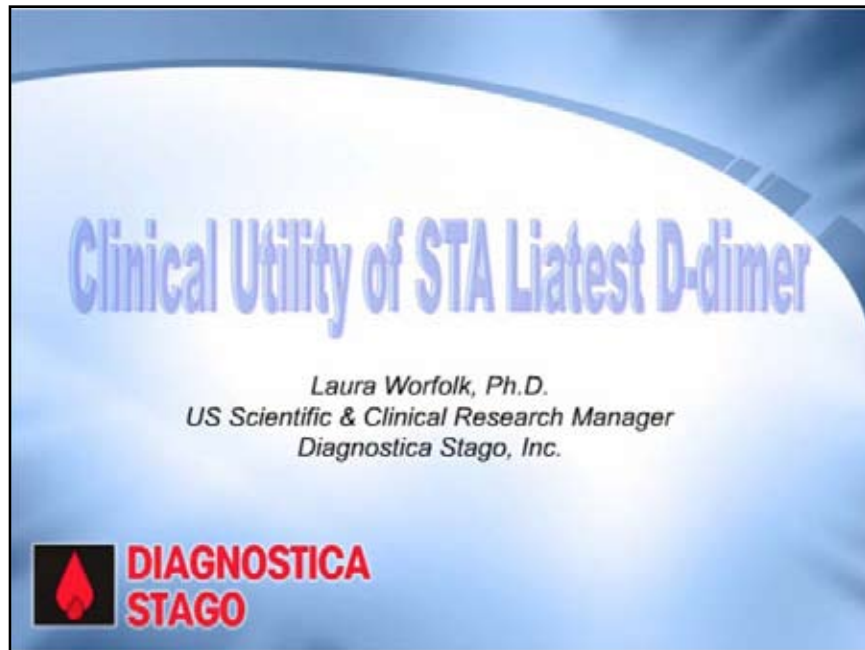


Clinical Utility of STA Liatest D-dimer

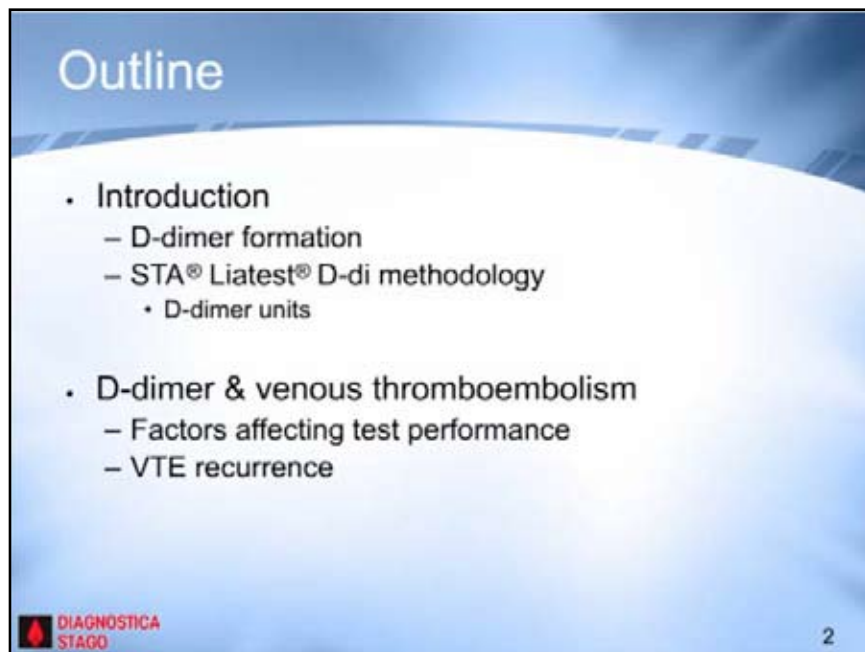
Laura Worfolk

Title: Clinical utility of STA Liatest D-dimer



Hello. My name is Laura Worfolk and I am the scientific and clinical research manager for Diagnostica Stago. It is my pleasure to provide this presentation titled Clinical Utility of the STA Liatest D-Dimer Assay.

Title: Outline



The outline of my presentation is summarized on this slide. I will provide a general overview of d-dimer formation and then discuss the different methodologies available for d-dimer measurement specifically focusing on the STA Liatest D-Dimer Assay. I will then discuss the clinical utility of the assay focusing on the evaluation of venous thromboembolism.

Title: What is D-dimer?

Outline

- Introduction
 - D-dimer formation
 - STA® Liatest® D-di methodology
 - D-dimer units
- D-dimer & venous thromboembolism
 - Factors affecting test performance
 - VTE recurrence

DIAGNOSTICA STAGO 2

So what is d-dimer? Well, it is simply a specific degradation product of plasmin cleaved cross-linked fibrin. In the following slides I have attempted to illustrate d-dimer formation in vivo, but please keep in mind that this is a very simplified and general overview.

Title: D-dimer formation

D-dimer Formation

Activation of Coagulation → Thrombin

Fibrinogen → Fibrinopeptide Release

Fibrin monomer (D-e-D-D-e-D)

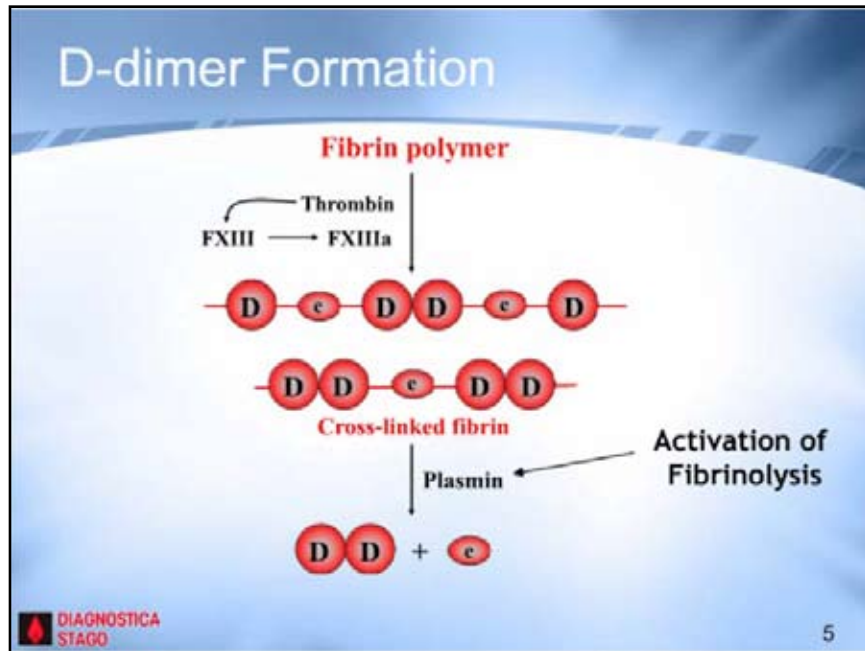
Fibrin polymer

DIAGNOSTICA STAGO 4

The pro-coagulant response culminates in the generation of thrombin. And as one of its many functions, thrombin cleaves fibrinogen, releasing fibrinopeptide A and B, and resulting in the formation of a fibrin monomer.

When the soluble fibrin monomers reach a certain concentration, they will polymerize spontaneously forming a large fibrin polymer meshwork. And this fibrin polymer or fibrin mesh then requires one more reaction to solidify the clot.

Title: D-dimer formation - Fibrin polymer

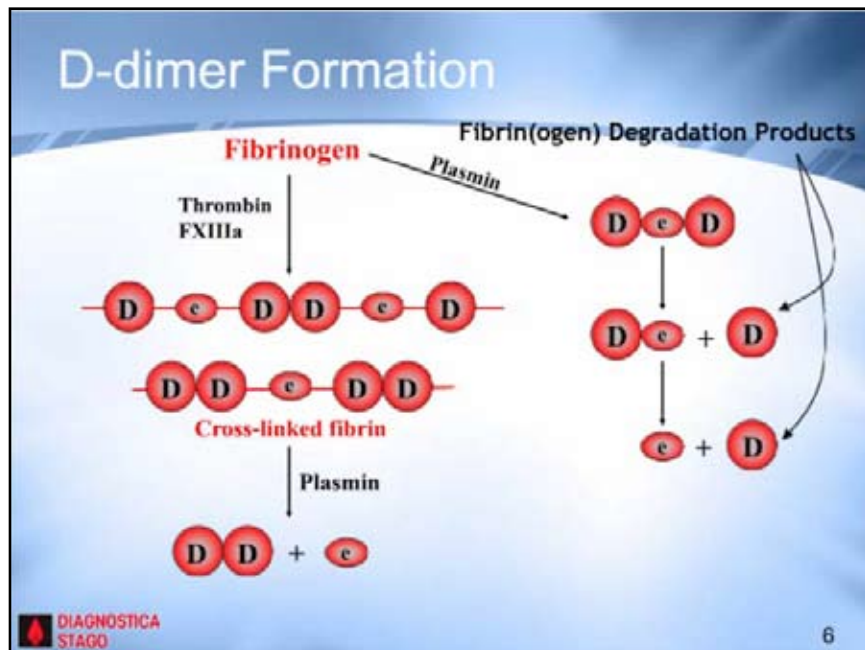


The final reaction also requires the activity of thrombin. Thrombin activates factor XIII and this activated factor then catalyzes a cross linking reaction between adjacent d-domains resulting in the formation of a covalent bond. The formation of this covalent bond results in a stable insoluble end product referred to as cross-linked fibrin.

However, once a stable clot is formed it must be removed to prevent occlusion of the vessel and to maintain hemostasis. And this process is called fibrinolysis. And the key enzyme involved in this pathway is called plasmin.

Plasmin will break down that clot yielding fibrin degradation products, but it can't break apart the bond that was formed by the action of factor XIIIa between the adjacent d-domains. So to summarize, d-dimer is a fibrin degradation product and its formation requires the activity of both thrombin and plasmin. And, therefore, detectable levels of d-dimer indicate that both the pro-coagulant and the fibrinolytic pathways have been activated.

Title: D-dimer formation - Fibrin(ogen) degradation products



Plasmin, however, is not specific for the cross-linked fibrin clot. It will also lyse fibrinogen as well, yielding fibrinogen degradation products.

However, d-dimer formation is unique to plasmin cleavage of the fibrin clot. It is not generated as a result of plasmin cleavage of fibrinogen because the cross linking reaction catalyzed by factor XIIIa has not occurred.

Laboratory Assays

- Qualitative
 - Latex agglutination
 - Red cell agglutination (*utilizes bispecific antibody*)
- Quantitative
 - ELISA
 - Immunoturbidimetric (=microlatex immunoassay)

DIAGNOSTICA STAGO 7

The laboratory assays that have been developed to detect d-dimer are summarized on this slide. Methodologies can be divided into two categories, qualitative and quantitative methods. The qualitative methods include latex and red cell agglutination. And because of the requirement for subjective interpretation, these assays in general lack the sensitivity required to assist in the exclusion of venous thromboembolism.

The quantitative assays include the ELISA assays and the immunoturbidimetric methods sometimes referred to as microlatex immunoassay. The ELISA assays are extremely sensitive and are often considered the gold standard for quantitative d-dimer testing.

However, the test is not suitable for a stat situation with a turnaround time of approximately two to three hours. In addition, the assay requires a plate reader and a lot of technical expertise.

The immunoturbidimetric methods for d-dimer are quantitative procedures performed on automated analyzers such as the Stago STAR evolution, STA, and STA compact. Monoclonal antibodies are coated to microlatex beads that have been designed specifically for the automated analyzers.

And these procedures are quick, they're cost effective, and they require no special equipment other than your routine coagulation analyzer. More importantly though, these assays have high sensitivity and can be used to assist in the diagnosis of venous thromboembolism.

Immunoturbidimetric Method

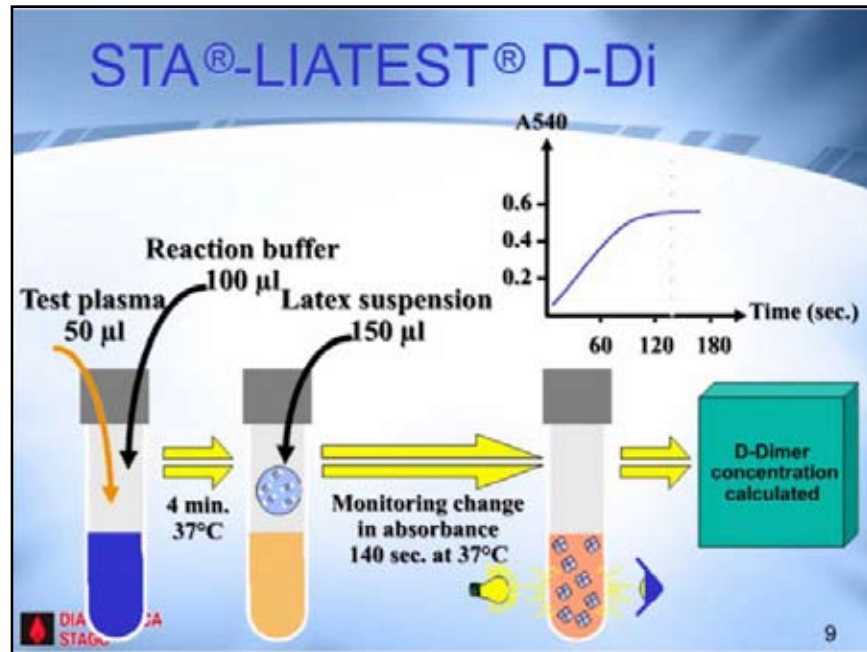
- Polystyrene antibody coated beads optimized for the STA® line of analyzers
- STA® LIATEST® D-DI
 - quantitative & sensitive
 - result in 7 minutes
 - assay range - 0.22-4 ug/mL FEU
 - automatic dilution of samples - 0.22-20 ug/mL
 - Precalibrated
 - 15-day on-board stability

DIAGNOSTICA STAGO 8

The STA Liatest d-di assay is an immunoturbidimetric assay. It is not a slide latex agglutination assay. It is for use on the STA line of instruments, as I've already mentioned, and it is a highly sensitive and quantitative assay with a quick turnaround time of approximately seven minutes. The assay range is from 0.22 to 4 $\mu\text{g/ml}$ FEUs which stands for fibrinogen equivalent units.

And with an additional sample dilution the working measuring range is extended to 20 micrograms per ml. The assay is precalibrated which eliminates the requirement and added expense of purchasing a standard.

Title: STA-Liatest D-Di



The principle of the STA Liatest d-di assay is illustrated on this slide. Test plasma is mixed with buffer, incubated, and then the reagent is added. If d-dimer is present in the sample, agglutination will result in the cuvette thereby changing the optical density of the solution.

The change in absorbance is monitored at 540 nanometers and the instrument reports out the final d-dimer concentration in fibrinogen equivalent units.

Title: D-dimer units

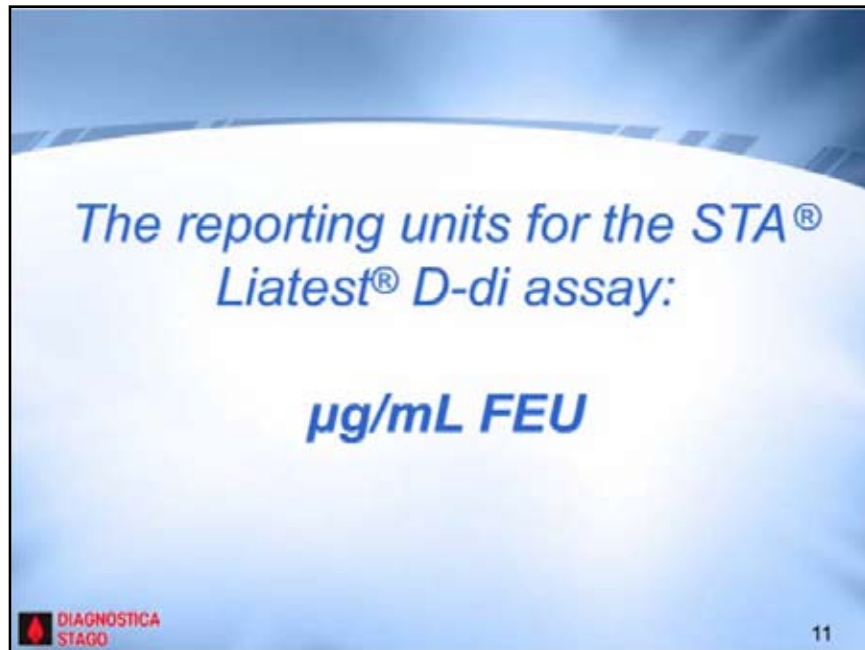
D-dimer Units

- D-dimer **D D**
 - based on weight of single D-dimer unit
- FEU (Fibrinogen Equivalent Units) **D e D**
 - based on the quantity of fibrinogen initially present that leads to the observed D-dimer
- To convert FEUs to D-dimer units, divide by 2
 - i.e. 1.0 $\mu\text{g/mL}$ FEU = 0.50 $\mu\text{g/mL}$ D-di

Now one of the most confusing areas related to d-dimer assays are the reporting units. Unfortunately, there is no international consensus on reporting units and they will vary from kit to kit. Now there are two types of units that are used by manufacturers, either d-di or fibrinogen equivalent units, abbreviated as FEU.

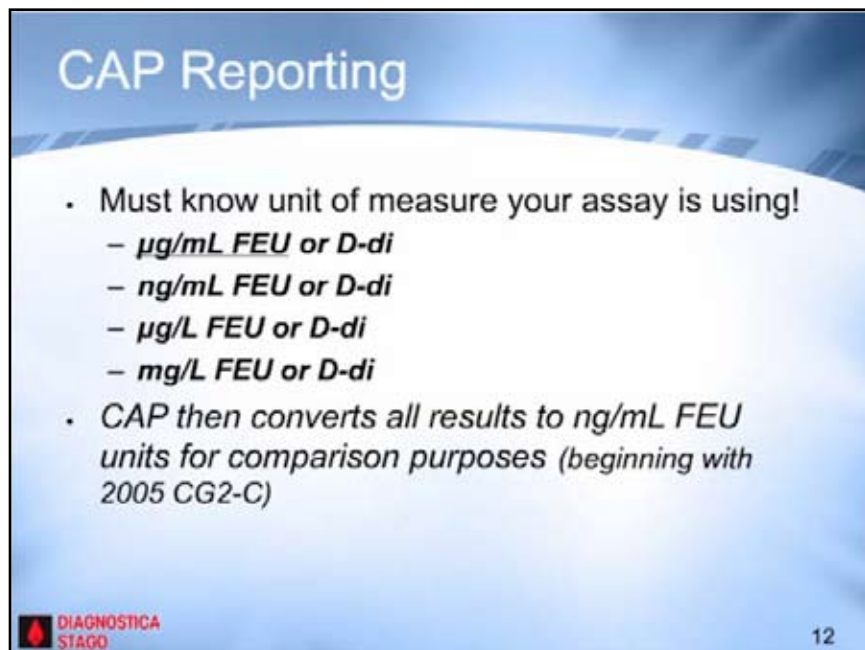
FEUs are based on the quantity of fibrinogen that's initially present that leads to the observed d-dimer. As the molecular weight of the e-domain is approximately equal to the d-dimer fragment, the molecular weight of one FEU is equal to two d-di units. Therefore, to convert FEUs to d-di, simply divide the result by two. But this is just an approximation.

Title: The reporting units for the STA Liatest D-di assay: $\mu\text{g/mL FEU}$



The reporting units for the Stago STA Liatest d-di assay are $\mu\text{g/ml FEUs}$. As part of ongoing quality assurance all laboratories must participate in some type of proficiency survey testing.

Title: CAP reporting

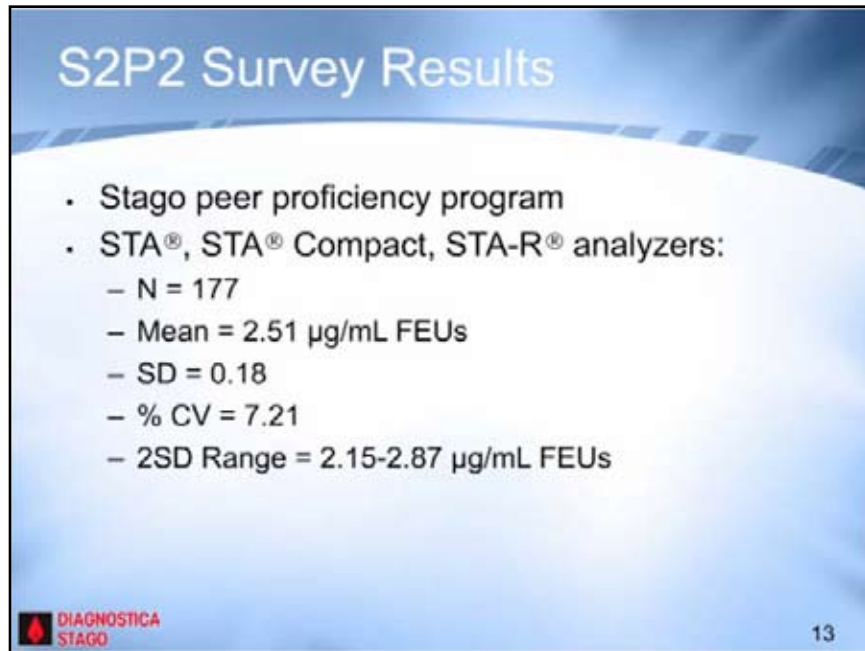


If your laboratory participates in the CAP survey which is by far the most frequently used program it is very important to be aware of how CAP handles d-dimer testing. When submitting results the lab must check off the appropriate units of their assay.

And I repeat, unless a change has been made to the test setup on the Stago analyzers, the reporting units for the STA Liatest d-di assay are $\mu\text{g/ml FEUs}$. And the various options of reporting units are shown on this slide.

And upon receiving the data, CAP will then convert all results to $\text{ng/ml fibrinogen equivalent units}$ for comparison purposes. Now in the past the STA Liatest d-dimer assay results have been reported in several different categories.

In order to make intralaboratory comparisons easier we ask our customers to select the immunoturbidimetric method and the Diagnostica Stago Liatest reagent.



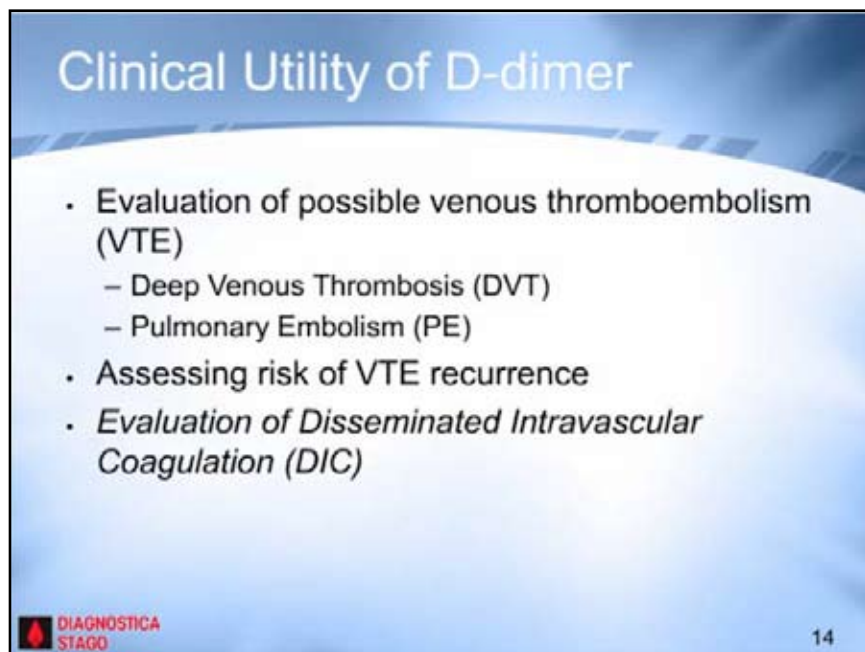
S2P2 Survey Results

- Stago peer proficiency program
- STA®, STA® Compact, STA-R® analyzers:
 - N = 177
 - Mean = 2.51 µg/mL FEUs
 - SD = 0.18
 - % CV = 7.21
 - 2SD Range = 2.15-2.87 µg/mL FEUs

DIAGNOSTICA STAGO 13

Because of the confusion associated with d-dimer reporting units, it is often difficult to assess intralaboratory precision using CAP survey data. One of the value added programs provided to our customers is the S2P2 program. Free of charge, twice a year samples are sent to participating labs for proficiency testing. D-dimer results are summarized on this slide.

One hundred seventy-seven labs responded with d-dimer results and the intralaboratory comparisons were quite good with a CV of approximately 7%.



Clinical Utility of D-dimer

- Evaluation of possible venous thromboembolism (VTE)
 - Deep Venous Thrombosis (DVT)
 - Pulmonary Embolism (PE)
- Assessing risk of VTE recurrence
- *Evaluation of Disseminated Intravascular Coagulation (DIC)*

DIAGNOSTICA STAGO 14

Now why is a d-di assay ordered? Well, by far, the most common reason is for the evaluation of a possible venous thromboembolism or VTE for short. More recent studies suggest that the assay may be useful in assessing the risk of VTE recurrence.

Historically, d-dimer was also ordered for the evaluation of disseminated intravascular coagulation. However, in this presentation I will only focus on the first two indications.

Venous Thromboembolism

- One disease entity with two patterns of clinical presentation:
 - Deep Venous Thrombosis (DVT)
 - Blood clot in leg veins
 - Affects ~ 2 million Americans/yr
 - Pulmonary Embolism (PE)
 - Clot migrates to the lung
 - Associated with significant morbidity & mortality

Anand et al., JAMA, 1998;279:1094-1099

DIAGNOSTICA STAGO 15

Venous thromboembolism is one disease entity with two patterns of clinical presentation, deep venous thrombosis or DVT for short and pulmonary embolism or PE for short. DVT usually presents as a clot in the leg and it affects approximately two million Americans per year.

If it is left untreated or it's not treated appropriately it may migrate to the lungs which is referred to as a PE and this can be fatal. The clinical diagnosis of DVT is unreliable and therefore requires objective testing.

It is estimated that approximately 75% of patients evaluated for suspicion of DVT have non-thrombotic causes of leg pain. Physicians don't want to treat everyone with anticoagulant therapy, since the therapy itself is associated with complications such as bleeding and heparin-induced thrombocytopenia.

Therefore, the challenge is to accurately diagnose so appropriate therapy can be started. Initiation of treatment in proximal DVT reduces the risk of fatal PE to less than 1%. The diagnosis of a DVT is difficult because the symptoms are nonspecific and that applies to the diagnosis of a PE as well.

Therefore, objective testing is required. Unfortunately, though, there's not one perfect test out there that is 100% sensitive and specific. So a simple inexpensive rapid and sensitive laboratory test would obviously be a great help.

D-dimer & VTE

- Almost all patients with acute disease (DVT or PE) have elevated D-dimer
- A positive D-dimer is not specific
- Patients with negative D-dimer unlikely to have acute VTE
- ***The greatest utility of D-dimer is its negative predictive value!***

DIAGNOSTICA STAGO 16

Where does d-dimer come into the picture? Well, almost all patients with acute disease will have an elevated d-di level. Unfortunately,

though, a positive d-di is not specific for VTE. There are many other situations that will yield an elevated d-dimer result.

The good news, though, is that for patients with a negative result it is extremely unlikely that they have had an acute VTE. Therefore, the power associated with this test is its negative predictive value.

In order to be useful for this application a sensitive and quantitated d-dimer assay should be used, one with documented high negative predictive value as well as high sensitivity.

Title: Useful definitions

Useful Definitions

- **Diagnostic Sensitivity ($TP/TP+FN$):**
 - ability of a test to detect disease
- **Diagnostic Specificity ($TN/TN+FP$):**
 - ability of a test to recognize *absence* of disease
- **Negative Predictive Value ($TN/TN+FN$):**
 - ability of test to identify disease-free individual among total population of patients with negative test result

DIAGNOSTICA STAGO 17

When the d-dimer assay is used to assist in the exclusion of a venous thromboembolism there are a few important definitions to keep in mind. Diagnostic sensitivity is defined as the ability of a test to detect disease whereas the diagnostic specificity is the ability of a test to recognize the absence of disease. Another important performance characteristic is the assay's negative predictive value.

And this is defined as the ability of a test to identify the disease free individual among the total population of patients with a negative test result. And I will discuss in more detail later on how different factors may affect the assay's sensitivity, specificity, and negative predictive value.

Title: D-di testing caveats

D-di Testing Caveats

- **Sensitive & quant. D-di assays have low specificity ($TN/TN+FP$)**
 - Elevated in many situations:
 - malignant growth
 - rheumatoid arthritis
 - intensive care patients
 - advancing age
 - prelude to DIC
 - inflammation
 - pregnancy

DIAGNOSTICA STAGO 18

Now why such low specificity? As I've already mentioned, there are many different situations that will result in an increased fibrin turnover and therefore a high d-di result. Other clinical situations that are associated with high d-dimer are listed on this slide.

Levels increase in pregnancy, cancers, trauma, extended bedrest, and as part of the normal aging process. Unfortunately, as we get older we all become more clottable.

Title: Testing caveats

Testing Caveats

- Factors that affect test performance:
 - Cut-off value
 - Patient population tested (incidence of disease)
 - Age of patient

DIAGNOSTICA STAGO 19

There are several important testing caveats to be aware of when d-dimer is used to assist in the exclusion of the diagnosis of VTE. The assay effectiveness is dependent on the prevalence of disease in the population and the cutoff value utilized by the laboratory. Another consideration that may affect assay performance is the age of the patient.

Title: Testing caveats continued...

Testing Caveats Continued...

- Cut-off value
 - Lower the cut-off:
 - higher sensitivity ($TP/TP+FN$)
 - lower specificity ($TN/TN+FP$)

The graph shows two overlapping normal distribution curves. The left curve is blue and labeled 'True Neg'. The right curve is red and labeled 'True Pos'. A vertical line labeled 'Cut-off Value' is positioned between the two curves. The area under the blue curve to the left of the cut-off is labeled 'False neg'. The area under the red curve to the right of the cut-off is labeled 'False pos'. The x-axis is labeled 'D-dimer Value'. The logo 'DIAGNOSTICA STAGO' and the number '20' are at the bottom.

The cutoff value is that value that separates the abnormal from the normal population. A perfect diagnostic test would clearly separate these two populations and yield 100% sensitivity and specificity. However, in reality a perfect diagnostic test does not exist and there is always some overlap.

By lowering the cutoff value it's easy to see that we can reduce the false negative rate and a higher sensitivity is obtained. However, lowering the cutoff value will also increase the false positive rate thereby decreasing the specificity of the assay.

Title: Cut-off value & test performance

Test	Cut-off (ug/mL)	Sensitivity	Specificity
Advanced	1.0	96.0	23.4
	1.1	96.0	29.5
	1.2	94.0	35.2
Stratus CS	0.3	96.0	38.9
	0.4	93.0	49.2
	0.5	92.0	55.3
Vidas New	0.3	98.0	21.3
	0.4	95.0	30.3
	0.5	93.0	41.8
STA Liatest	0.3	97.0	23.0
	0.4	95.0	41.0
	0.5	94.0	48.0

DIAGNOSTICA STAGO Thrombosis Research 112 (2003) 25-32 21

An example of how the cutoff value impacts assay sensitivity and specificity is shown on slide 21. In this study the sensitivity and specificity of four different assays was calculated at different cutoff levels.

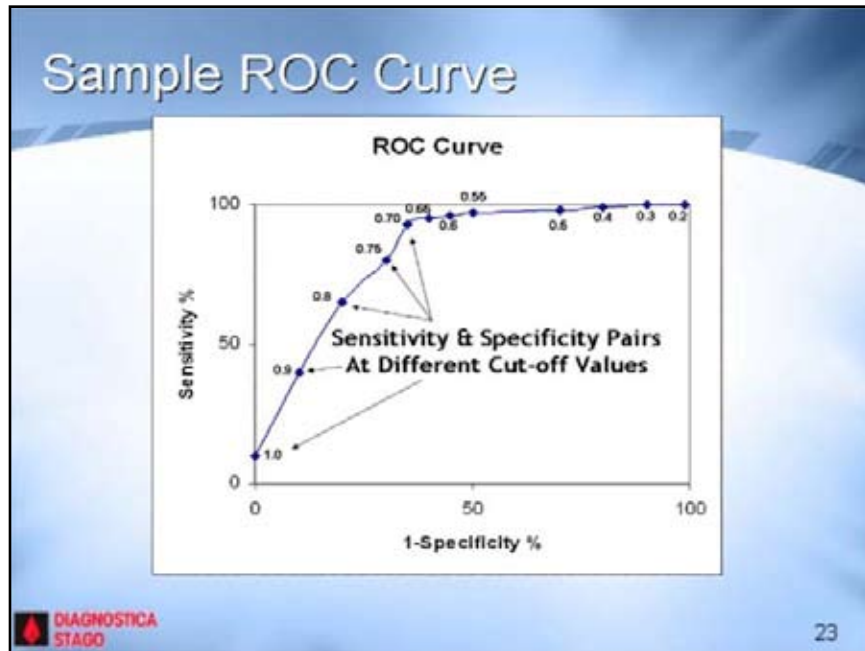
For all assays evaluated decreasing the cutoff value increased the sensitivity but at the same time decreased the specificity. And this was due to a decrease in the false negative rate and an increase in the false positive rate.

Title: Establishing cut-off value

-
- Cut-off may be based on desired sensitivity or specificity
 - One method = Receiver Operator Characteristic Analysis (ROC)
 - Test normal & patient samples
 - Compare to diagnostic standard in use
 - ROC curve
- DIAGNOSTICA STAGO 22

Now how is that cutoff value determined? The cutoff values may be based on the desired sensitivity or specificity. One method for determining the appropriate cutoff value is called receiver operator characteristic analysis. ROC analysis assesses the value of a quantitative test in terms of sensitivity, specificity, and predictive values.

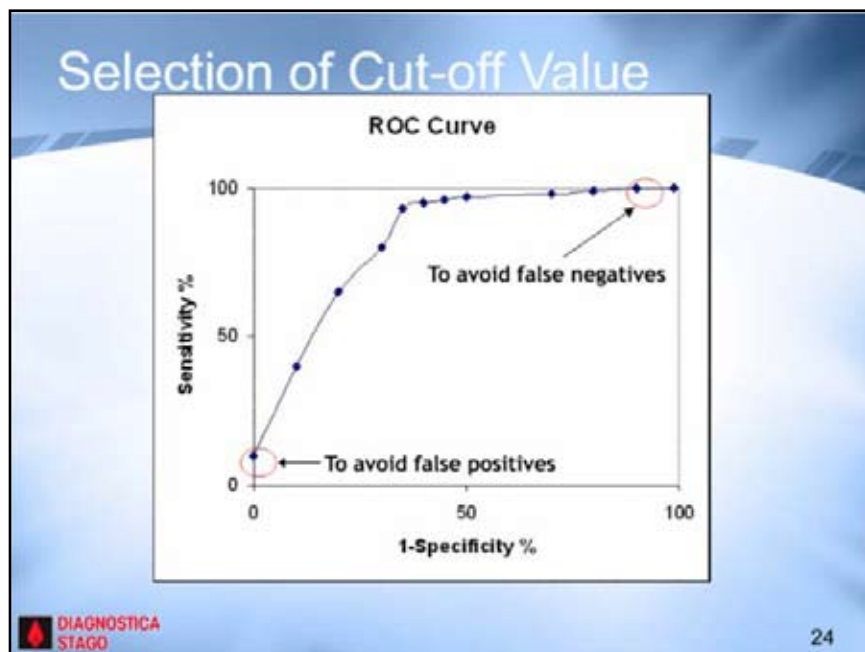
Title: Sample ROC curve



A sample ROC curve is shown on this slide. In this type of analysis d-di testing is performed using patient samples undergoing evaluation for suspicion of VTE.

Results of d-di testing are then compared to objective test results such as compression ultrasound and the sensitivity and specificity are calculated at different cutoff values. An ROC curve is generated which plots the sensitivity on the Y axis and one minus the specificity on the X axis. Each data pair represents a different cutoff value.

Title: Selection of cut-off value



Now a perfect diagnostic test would have a curve in the upper left hand corner with 100% sensitivity and specificity.

A straight diagonal line going through the lower left to the upper right hand side of the graph represents a useless test that cannot discriminate between normal and abnormal samples. The area under the curve may be used to assess the accuracy of the diagnostics test. And the larger the area, the better the test.

When the d-di assay is used to assist in the diagnosis of a DVT or PE, a false negative result may have more serious implications than a false positive result. And with a false negative result, the possibility exists that a patient may be sent home untreated.

Therefore, if false negatives are more undesirable, a cutoff value in the upper right hand corner of the curve should be chosen where 100% sensitivity is obtained. Now from a practical point of view not many laboratories have the number of specimens required to

perform ROC analysis to establish a cutoff value.

Numerous published studies have been performed with the STA Liatest d-dimer assay, and the data suggest that a cutoff value in the range of 0.4 to 0.5 mics per ml is optimal. However, validation of a cutoff value is recommended before going live with the new assay.

If ROC analysis is not feasible one suggestion is to collect samples from patients undergoing an evaluation for suspicion of VTE and compare the d-di results to the objective test findings. And based on this analysis the cutoff value may be adjusted as needed.

Title: Testing caveats

Testing Caveats

- **Factors that affect test performance:**
 - Cut-off value
 - Patient population tested (incidence of disease)
 - Age of patient

DIAGNOSTICA STAGO 25

The next testing caveat I will discuss is how the incidence of disease affects test performance. And when I refer to incidence of disease I refer to the incidence of VTE.

Title: Testing caveats continued

Testing Caveats Continued

- **Patient population tested (incidence of disease)**
 - *Low incidence (generally outpatient population):*
 - higher NPV ($TN/TN+FN$) & specificity ($TN/TN+FP$)
 - *High incidence (generally inpatient population):*
 - lower NPV & specificity

DIAGNOSTICA STAGO 26

In general the lower the incidence of disease in a population, the higher the negative predictive value and the specificity. On the other hand, a population with a high incidence will have a much lower negative predictive value and specificity. And an example of this is shown on the next slide.

Patient Population & Performance

- Comparison of diagnostic accuracies in outpatients & hospitalized patients for PE:
 - Outpatient: **NPV** **Specificity**
 - Liatest 98% 48%
 - Vidas 99% 51%
 - Inpatient: **NPV** **Specificity**
 - Liatest 91% 20%
 - Vidas 93% 20%

Clinical Chemistry 49:1483-1490, 2003

DIAGNOSTICA STAGO 27

In this study the diagnostic accuracies of two quantitative assays were assessed in an inpatient and outpatient population. All patients were being evaluated for suspicion of PE. For both assays the negative predictive value and the specificity yielded was higher in the outpatient population in comparison to the inpatient population.

One possible explanation for these results is that the inpatient population may have comorbid conditions resulting in an elevated d-di level unrelated to venous thromboembolism. These results suggest that the clinical utility of a sensitive and quantitative assay may be greater in the outpatient population.

Testing Caveats

- Factors that affect test performance:
 - Cut-off value
 - Patient population tested (incidence of disease)
 - Age of patient

DIAGNOSTICA STAGO 28

The next testing caveat that I will focus on is how patient age may impact assay performance.

Title: Testing caveats - Patient age

Testing Caveats

- **Patient Age:** D-dimer levels increase with age. Should there be age specific cut-off levels?
- D-di & PE study →
- **Conclusion:** increase in cut-off yields unacceptable rate of false negative results

Cut-off ug/L	60-70 yr Sens./Spec.	>70 yrs Sens./Spec.
500	99.5/26.8	99.3/18.5
600	96.7/33.9	96.6/23.2
700	95.4/39.6	95.9/28.9
800	94.4/49.1	95.3/40.7
1000	92.1/61.9	94.6/56.9

Thromb Haemost 2001;85:744

DIAGNOSTICA STAGO 29

Studies have revealed that d-dimer levels increase with age. In order to obtain optimal sensitivity and specificity for all age groups, should we have age-specific cutoff ranges? And this question was addressed in a study published several years ago. In this study patients were evaluated for suspicion of a PE.

At the completion of the study the patients were separated into specific age groups and the sensitivity and specificity calculated at different cutoff levels. As the cutoff value was raised, there was an increase in specificity; however, the sensitivity decreased as well.

These investigators concluded that although the specificity was increased with increasing cutoff value and theoretically that would increase the clinical utility of the assay that the simultaneous loss in sensitivity was unacceptable.

Title: Diagnostic strategy for VTE

Diagnostic Strategy for VTE

- Quantitative & sensitive D-dimer testing
- Objective testing
 - i.e. CUS, V/Q Scanning, Venography
- Clinical assessment (*scoring system*)
 - Signs & symptoms of DVT
 - Risk factors for DVT
 - Potential alternative diagnosis
 - Used to calculate *pre-test probability of disease*
 - Stratifies patients into low, moderate or high categories

DIAGNOSTICA STAGO 30


The diagnostic strategy for VTE may utilize a combination test approach including quantitative d-dimer testing, objective testing, and clinical assessment. A clinical scoring system that was first described by Dr. Wells and colleagues may be used to calculate the pre-test probability of disease.

It consists of a well-defined set of questions with an associated score that are geared to assess the likelihood of a DVT or an alternative diagnosis. The score is then used to categorize the patients into three groups: low, moderate, and high pre-test probability.

Title: Clinical model for predicting DVT

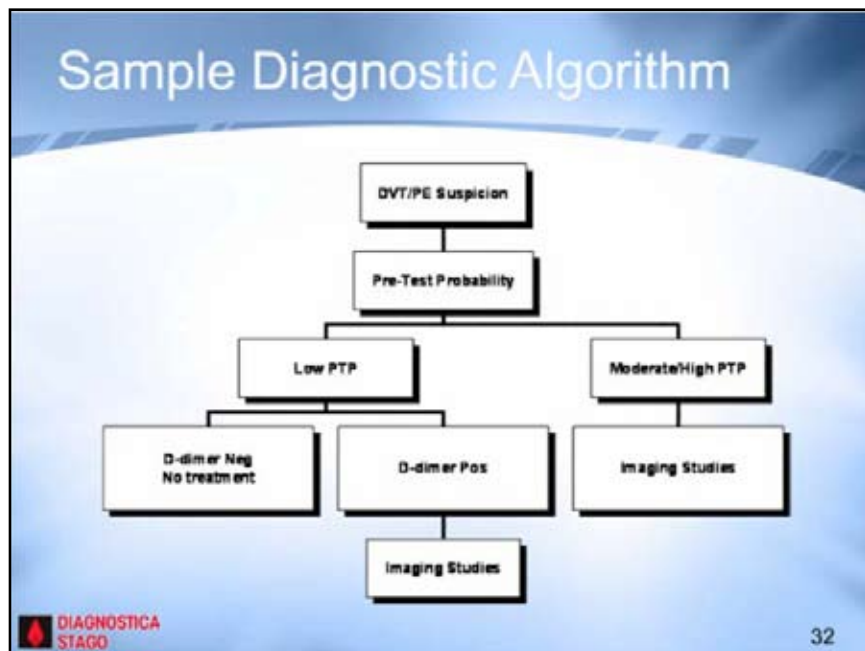
Clinical Model for Predicting DVT

Pre-Test Probability Questionnaire	Score
Active cancer	1
Paralysis, paresis or recent plaster immobilization of lower extremities	1
Recently bedridden > 3d or major surgery w/in 4 wk	1
Localized tenderness along the distribution of deep venous system	1
Entire leg swollen	1
Calf swelling 3 cm > asymptomatic side (measured 10 cm below tibial tuberosity)	1
Pitting edema confined to the symptomatic leg	1
Collateral superficial veins (nonvaricose)	1
Alternative diagnosis as likely or greater than that of DVT	-2

 Wells, et al. Lancet, 1997;350:1795-1798 31

For reference purposes the pre-test probability questionnaire is shown on slide 31. How are sensitive d-dimer testing, clinical assessment, and objective testing incorporated into the diagnostic strategy? An example of a diagnostic algorithm is shown on slide 32.

Title: Sample diagnostic algorithm




In this algorithm, when there is suspicion of VTE the pre-test probability is assessed. Those patients that fall in the low category have a sensitive quantitative d-dimer test performed. If the result is negative treatment is withheld. If it is positive the appropriate imaging studies are performed.

The moderate to high pre-test probability patients are also evaluated with imaging studies. Please keep in mind that this is just one example of a diagnostic algorithm and the approach to making the diagnosis will be unique for each institution.

STA Liatest D-dimer & VTE Studies

Clinical Context	# of Patients	Reference Standard	% VTE	D-di Cut-off (ug/mL FEU)	Performance Characteristics (95% CI)		
					Sens.	NPV	Spes.
DVT	300 Outpatients	N/A. A clinical management study, negative Liatest D-dimer assay used to withhold treatment. Therefore, performance characteristics not provided. 1 of 134 negative D-di patients developed VTE during follow-up. (D 7251, 9276; CI 0.02-4.039%) ¹					
FE	55 Outpatients	Various ²	69	0.4	100 (91-100)	Not calculated (NC)	
DVT/PE	118 Outpatients	Various ³	36	0.5	95 (84-98)	NC	43 (31-55)
FE	234 Outpatients	Various ⁴	8	0.5	99 (87-98)	98 (93-100)	48 (41-55)
DVT	344 Outpatients	CISS	29	0.5	94 (87-98)	95 (90-98)	48 (42-54)
DVT	537 Outpatients	CISS ⁵	42	0.5	98 (95-99)	95 (89-99)	32 (27-37)
DVT	99 Outpatients	US ⁶	2	0.5	100 ⁷	100 ⁷	53 ⁷
DVT	134 Outpatients	CUS	19	0.4	100 (82-100)	100 (85-100)	31 (23-41)
DVT	99 Outpatients	Venography	30	0.5	96 (86-100)	92 (74-99)	47 (32-62)
DVT/PE	123	Various ⁸	42	0.5	96 (86-100)	93 (78-99)	39 (28-51)
DVT	67 Patients with Paraplegia or Tetraplegia	US ⁹	9	0.5	NC	100 ⁷	NC
FE	501 Outpatients	Various ¹⁰	30	0.5	99 (96-100)	99 (96-100)	43 (38-47)
FE	336 Outpatients	Various ¹¹	38	0.5	100 (97-100)	100 (94-100)	39 (29-41)
FE	251	Perfusion Pulmonary angiography	19	0.5	94 ¹²	94 ¹²	38 ¹²
FE	142 ¹³	Various ¹⁴	42	0.5	93 (84-98)	90 (76-97)	43 (32-54)
DVT	464 Outpatients	Various ¹⁵	44	0.48	99 ¹⁶	94 ¹⁶	48 ¹⁶


 33

Numerous studies incorporating clinical assessment, objective testing, and the STA Liatest d-di assay have been published. A summary of the findings are shown on slide 33. References can be requested by visiting the Diagnostica Stago Web site. The Web site address will be shown at the end of this presentation.

D-dimer & Recurrent DVT

- Recurrent DVT occurs in up to 1/3 of patients, despite anticoagulant treatment
- Clinical dx inaccurate for differentiating recurrent DVT from post-thrombotic syndrome or other causes of leg pain
- Many limitations associated w/ tests for dx recurrent DVT
- *What is the value of D-dimer testing?*

Rathbun et al. Ann Intern Med. 2004;141:839-845

 34

Unfortunately, once a patient has deep venous thrombosis they are at an increased risk for another event despite standard oral anticoagulant therapy. As a diagnosis of recurrent DVT is difficult to differentiate from post-thrombotic syndrome, the question that arises is d-dimer testing of any value in this situation.

D-di & Recurrent DVT

- Prospective cohort study (*w/ 3 month follow-up*)
 - Consecutive patients w/ history of DVT (n=300)
 - STA® Liatest® D-di testing performed
 - Neg D-di: *heparin withheld or withdrawn*
 - Results:
 - 45% of patients negative D-di at presentation
 - Low incidence of confirmed DVT in group with neg D-di, 0.75% (CI 0.02-4.09%)
 - “*STA Liatest assay provides a simple method w/ high clinical utility to exclude acute recurrent DVT.....*”

DIAGNOSTICA STAGO Rathbun et al. Ann Intern Med. 2004;141:839-845 35

In a prospective cohort study published by Dr. Rathburn and coworkers, the usefulness of d-dimer testing to exclude recurring DVT was assessed. If the STA Liatest d-di value was negative, which means it was below the cutoff value, patients were not treated.

If the value was above the cutoff, patients underwent additional diagnostic testing. As there was less than a 1% DVT rate in the d-dimer negative group, the authors concluded that the assay had high clinical utility to exclude the diagnosis of recurrent DVT.

Assessing Risk of Recurrence

- Persistent high D-dimer levels associated with increased risk of VTE recurrence
 - Prospective study, n=396 patients
 - D-di level measured multiple times after therapy discontinued
 - *D-DI has high NPV for VTE after therapy discontinued*

DIAGNOSTICA STAGO Palareti, Legnani et al, T&H 2002;87:7-12 36

What about assessing the risk of recurrence? Is the assay of any value in detecting those patients who might have another event? Studies have demonstrated that those patients with a consistent elevated d-dimer value are at a high risk for recurrence.

Palareti and coworkers reported that d-di levels assessed after discontinuation of oral anticoagulant therapy were useful in excluding the diagnosis of recurring DVT. In other words, the assay had high negative predictive value. These are findings that need to be confirmed with further prospective studies.

Title: Is high D-dimer predictive of VTE?

Is High D-dimer Predictive of VTE?

- Prospective study, 1528 non-selected patients w/ PE suspicion
 - D-dimer (n=923) & pre-test probability assessed
 - Negative D-di: ruled out dx
 - Positive D-di: patients underwent CUS, lung scan or helical CT
 - Results:
 - Overall PE prevalence 19.9%
 - D-di sensitivity = 97.4%, specificity = 56.2%
 - PE prevalence increased with STA® Lia D-di levels (D-DI > 2 independently associated with PE)

Bosson et al. J Thromb & Haemost 2005;3:93-99

DIAGNOSTICA STAGO 37

Throughout this presentation I have focused on the ability of a sensitive d-dimer assay to exclude the diagnosis of VTE. A slightly different question was addressed by Bresson and colleagues this year.

Are high d-dimer values predictive of VTE for the first time event? What is the positive predictive value of the assay? And in their study of over 900 patients evaluated for PE suspicion an STA Liatest d-dimer value greater than two mic per ml FEU was independently associated with PE. Further studies are needed to confirm this interesting finding.

Title: Summary

Summary

- Sensitive D-dimer assays can be used to assist in DVT/PE exclusion (& values may be predictive)
- Test performance is highly dependent on:
 - Test cut-off
 - Patient population evaluating
 - Patient age & anticoagulation status
- D-dimer may be useful for assessing risk of recurrence

DIAGNOSTICA STAGO 38

In summary I hope I have provided useful information on how sensitive d-dimer assays may be used to assist in venous thromboembolism exclusion.

Now there are numerous factors that will affect test performance and it is important to be aware of these factors so that the proper information can be shared with the ordering physicians. Last, in the future we may see further applications of the assay for assessing risk of recurrence and maybe even utilizing the assay as a positive predictor.

Title: To request additional information or references, please visit www.stago-us.com



I thank you for your attention. To request any of the references cited, please visit the Diagnostica Stago Web site at www.stago-us.com. Thank you very much.