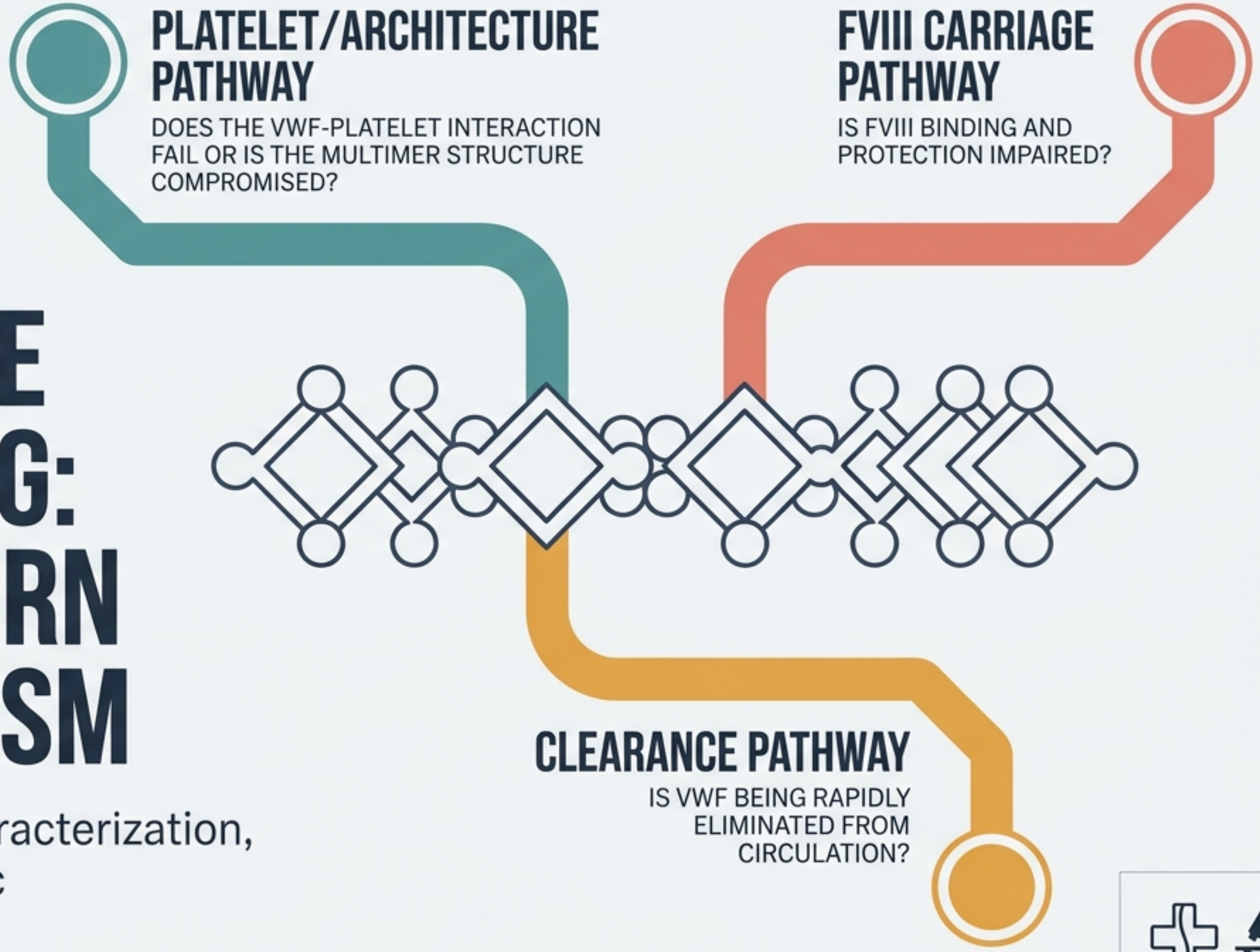
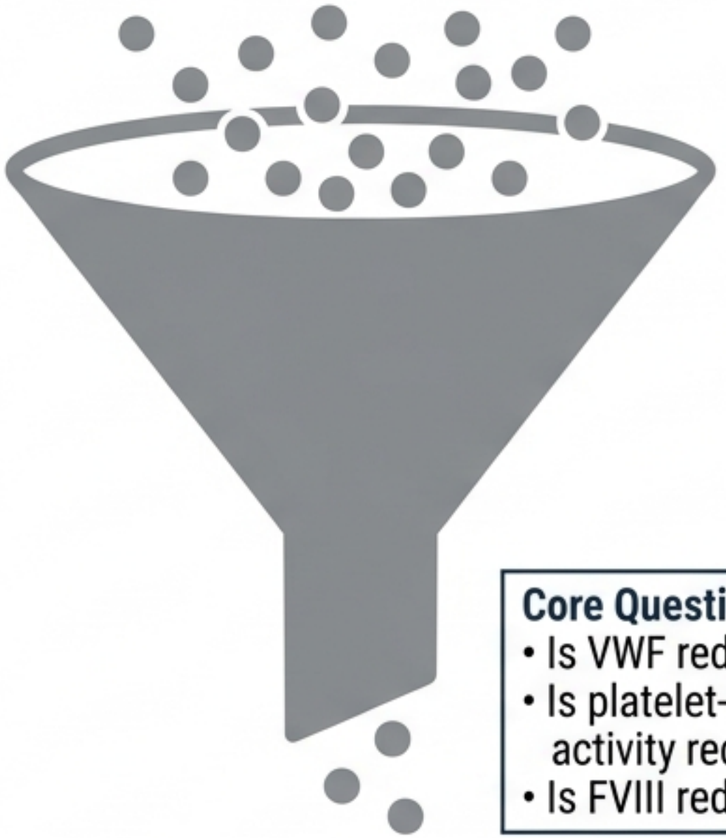
SECOND-LINE VWD TESTING: FROM PATTERN TO MECHANISM

A visual framework for characterization, localization, and diagnostic boundary adjudication.



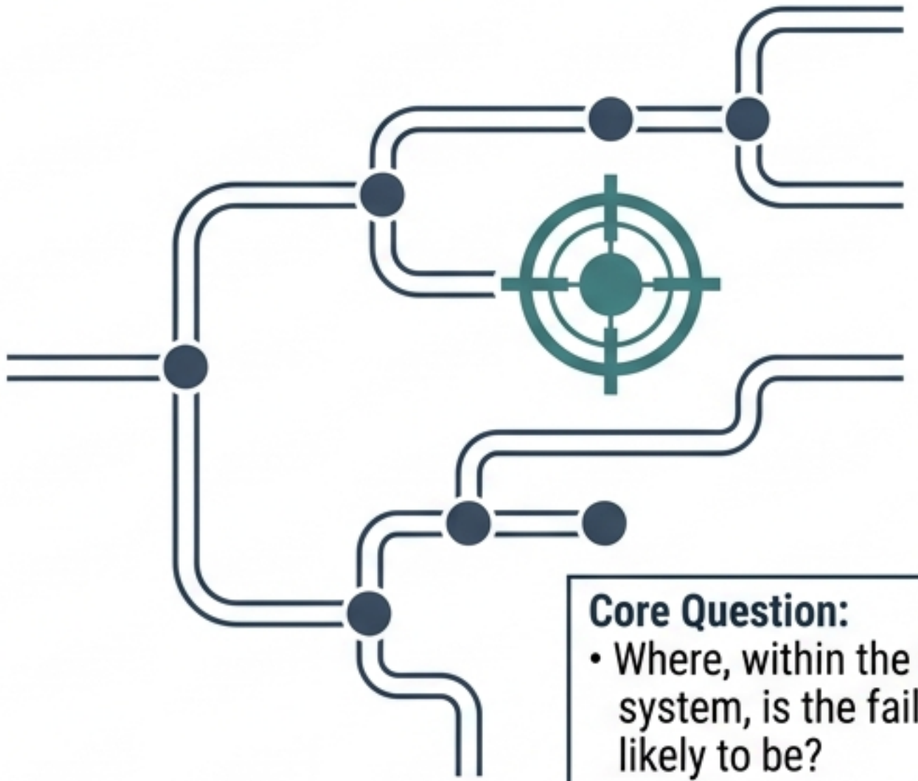
FIRST-LINE: PATTERN RECOGNITION



Core Questions:
- Is VWF reduced?
- Is platelet-dependent activity reduced?
- Is FVIII reduced?

Goal: Identify the clinical and laboratory pattern.

SECOND-LINE: MECHANISTIC LOCALIZATION



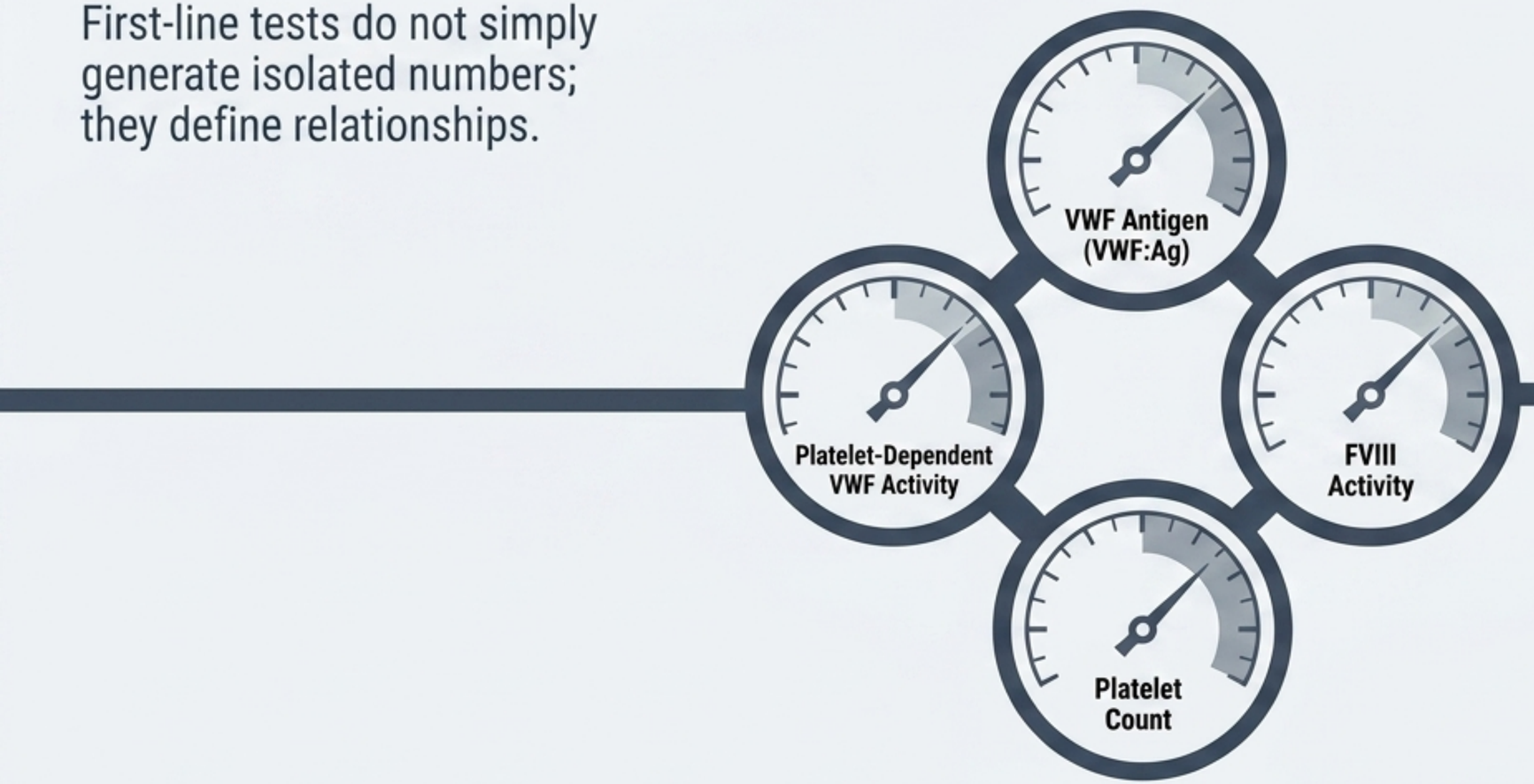
Core Question:
- Where, within the VWF system, is the failure likely to be?

Goal: Characterize the specific molecular or structural defect.

Second-line testing is not "screening harder." It is the next step only when a first-line pattern creates a specific mechanistic question.

THE FOUNDATION: FIRST-LINE TESTS DEFINE RELATIONSHIPS

First-line tests do not simply generate isolated numbers; they define relationships.



Path A: Proportional reduction = Quantitative problem.

Path B: Disproportionate reduction = Qualitative problem.

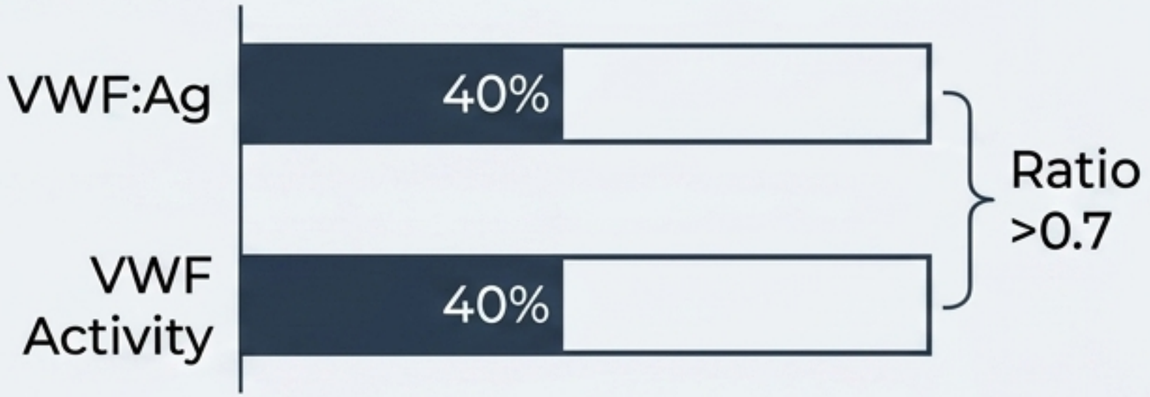
Path C: Low platelet count / Unusually low FVIII = The diagnostic map changes entirely.

⚠ **If the pattern is borderline, unstable, or biologically implausible, repeat first-line testing under better conditions before ordering specialized assays.**

THE BRIDGE: VISUALIZING THE ACTIVITY-TO-ANTIGEN RATIO

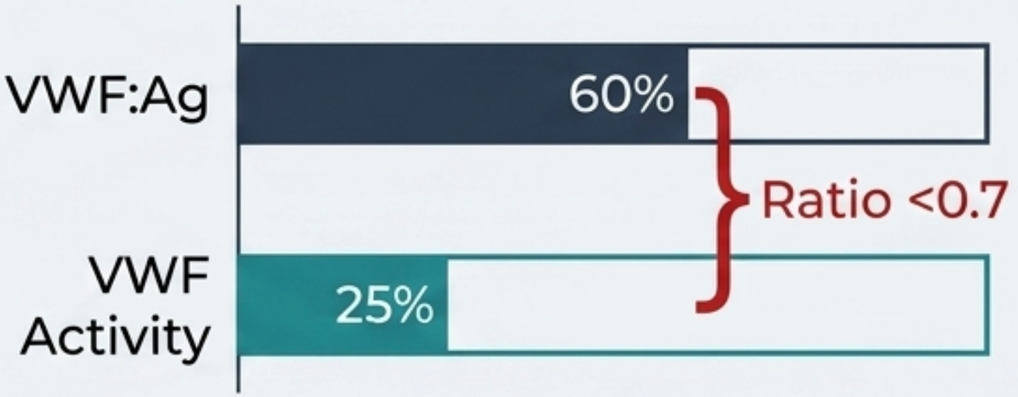
The **<0.7 cutoff** is a directional clue, not a diagnosis. It bridges the gap between first-line screening and second-line characterization by asking: Is there a qualitative defect in VWF function?

PROPORTIONAL (Type 1 Pattern)



The amount of protein is reduced, but the protein present functions normally.

DISPROPORTIONATE (Type 2 Pattern)



The activity falls out of proportion to the antigen. The molecule is present but qualitatively impaired.

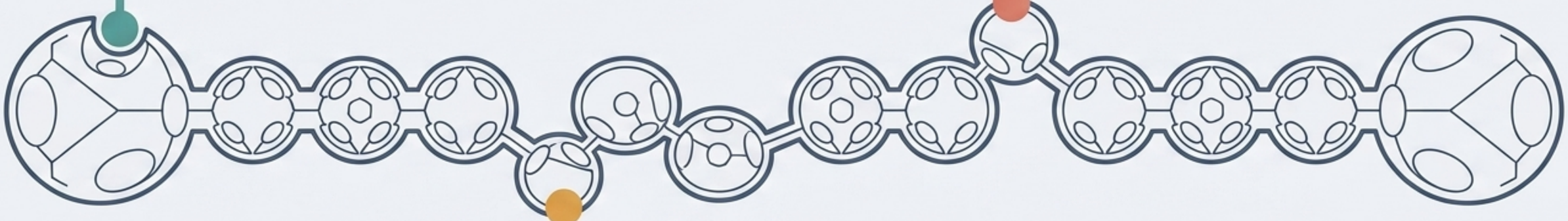
LOCALIZING THE VWF SYSTEM

PLATELET BINDING SITE (GPIb)

Evaluated by Platelet-binding assays / RIPA

FVIII BINDING SITE

Evaluated by VWF:FVIIIB



COLLAGEN BINDING SITE

Evaluated by VWF:CB

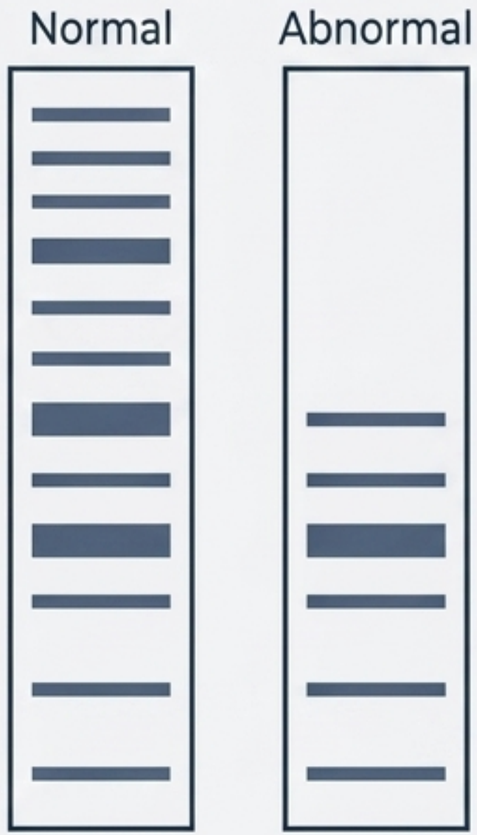
MULTIMER ARCHITECTURE / SIZE

Evaluated by Multimer Analysis

KEY PRINCIPLE: Do not order a second-line test until you can say WHERE on this molecule it is looking.

FORK 1: MULTIMER ARCHITECTURE

ARCHITECTURE NODE



CLINICAL PATTERN

Disproportionately low platelet-dependent VWF activity (Activity/Ag ratio <0.7).

MECHANISTIC QUESTION

Is the VWF architecture abnormal?

TARGETED TEST

Multimer Analysis.

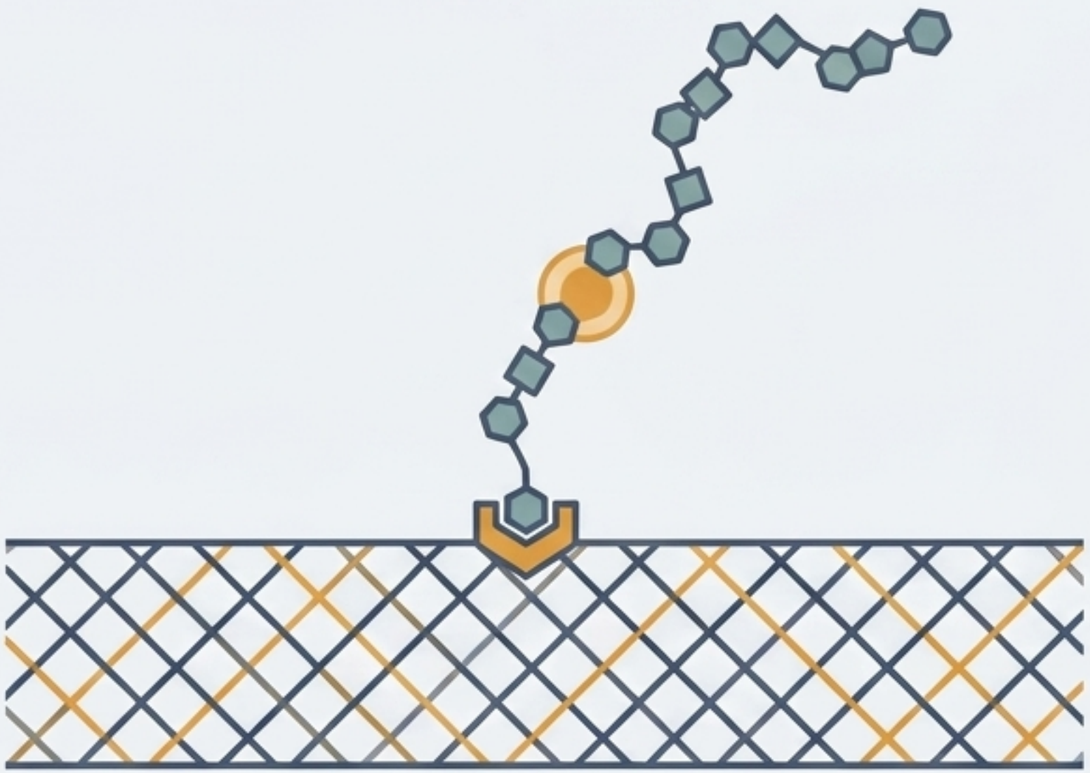
THE BOUNDARY ADJUDICATED

- Loss of HMW multimers supports Type 2A or Type 2B VWD.
- Preserved multimers (despite low activity) supports Type 2M VWD.

Clinical Nuance: Multimer analysis reveals architecture, not bleeding severity. It does not replace a bleeding history.

FORK 2: VESSEL-WALL ADHESION

COLLAGEN ADHESION NODE



CLINICAL PATTERN

Suspected loss of HMW multimers or an isolated qualitative defect.

MECHANISTIC QUESTION

Can VWF bind subendothelial collagen appropriately?

TARGETED TEST

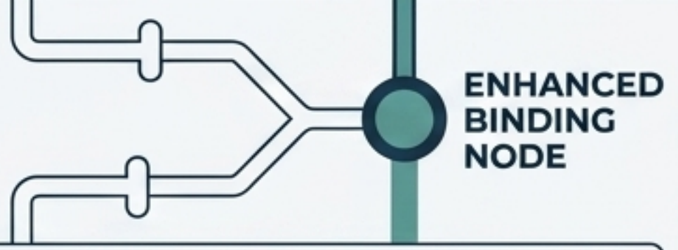
VWF Collagen-Binding Assay (VWF:CB) / VWF:Ag ratio.

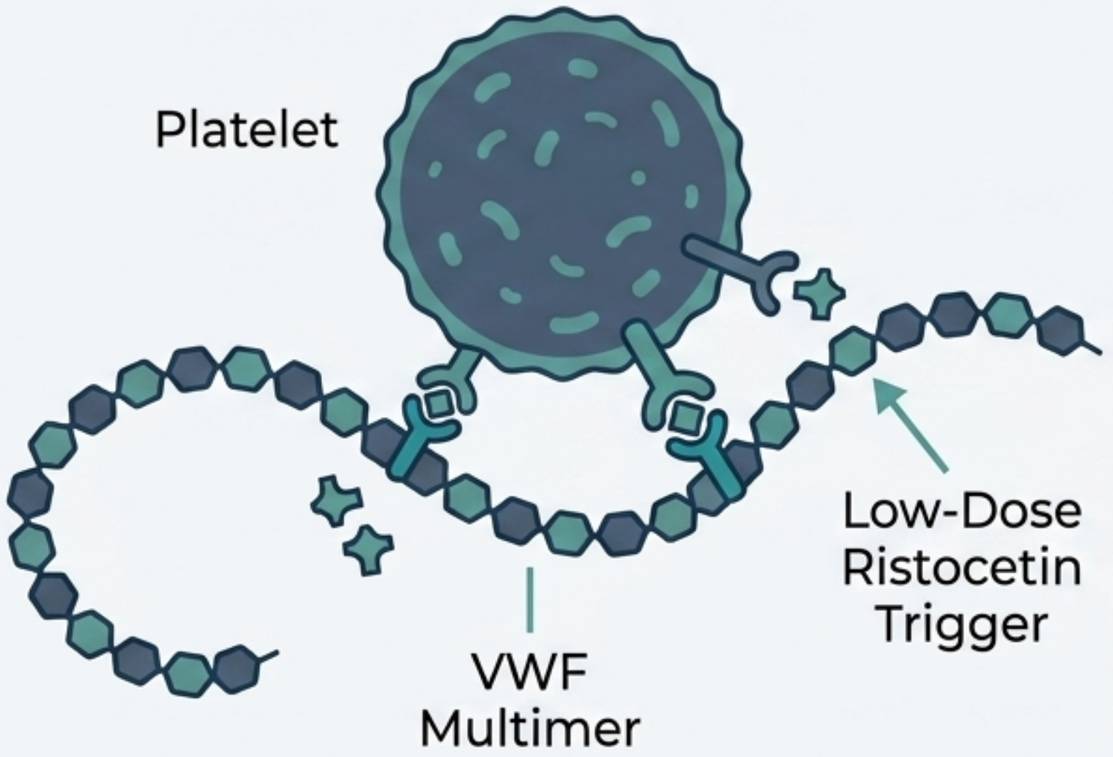
THE BOUNDARY ADJUDICATED

- Low VWF:CB/VWF:Ag correlates with the loss of HMW multimers (Types 2A/2B).
- Preserved VWF:CB with low platelet activity supports a Type 2M pattern.

Clinical Nuance: VWF:CB is an excellent functional surrogate for HMW multimer loss, but its diagnostic performance depends heavily on laboratory optimization and the collagen source (Type I/III) used.

FORK 3: ENHANCED PLATELET BINDING





CLINICAL PATTERN

Disproportionately low activity + loss of HMW multimers + unexpected or stress-related Thrombocytopenia

MECHANISTIC QUESTION

Is platelet agglutination occurring at lower ristocetin concentrations than expected?

TARGETED TESTS

Low-dose RIPA & Targeted Genetic Testing.

THE BOUNDARY ADJUDICATED

- Defect in VWF = Type 2B VWD.
- Defect in the platelet receptor = Platelet-Type VWD.

Clinical Nuance: While low-dose RIPA is mechanistically central, modern diagnostic guidelines increasingly favor targeted genetic testing to definitively separate 2B from Platelet-Type VWD.

FORK 4: FVIII CARRIAGE

CLINICAL PATTERN

FVIII is disproportionately lower than expected for the patient's VWF:Ag and activity levels.

MECHANISTIC QUESTION

Is the problem VWF-mediated FVIII carriage, or is this primary FVIII deficiency?

TARGETED TESTS

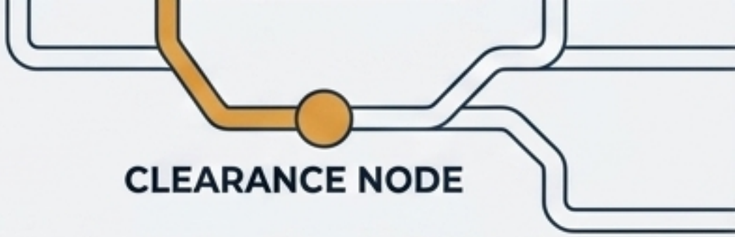
VWF:FVIIIB (FVIII binding assay) & Targeted Genetic Testing.

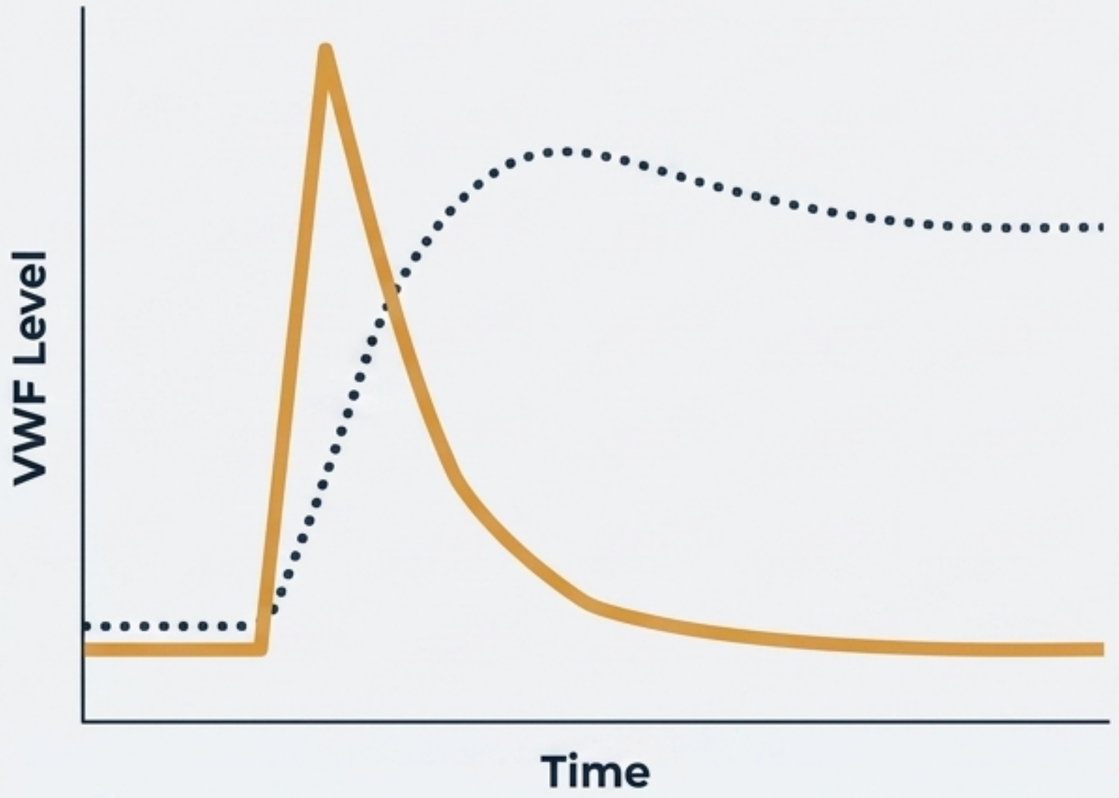
THE BOUNDARY ADJUDICATED

- Impaired VWF binding to FVIII = Type 2N VWD.
- Primary FVIII deficiency = Mild Hemophilia A.

Clinical Nuance: This distinction is not semantic. It radically alters inheritance counseling, treatment plans, and how family histories are interpreted.

FORK 5: ACCELERATED CLEARANCE





CLINICAL PATTERN

VWF is repeatedly low, or the patient exhibits a short-lived clinical correction to therapy.

MECHANISTIC QUESTION

Is the low VWF signal due to accelerated clearance and shortened survival rather than low production alone?

TARGETED TESTS

VWF Propeptide (VWFpp)/VWF:Ag ratio; DDAVP trial with 1-hour and 4-hour post-infusion testing.

THE BOUNDARY ADJUDICATED

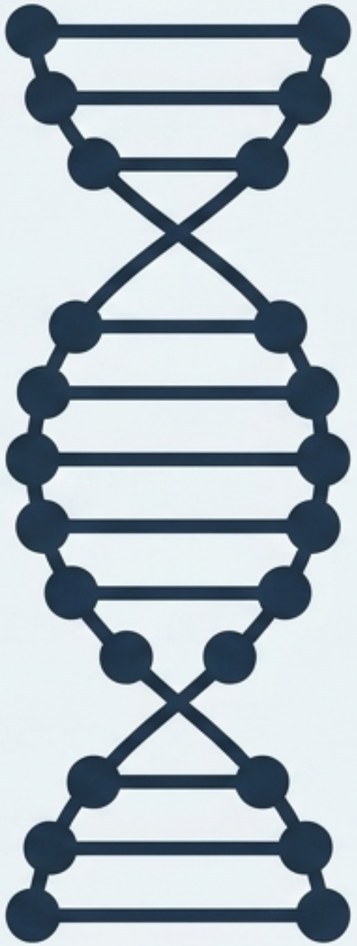
- Identifies Type 1C VWD or other increased-clearance phenotypes.

Clinical Nuance: VWF propeptide and mature VWF are secreted together but cleared at different rates. An increased ratio proves the protein is vanishing too quickly.

GENETIC TESTING: WHEN PLASMA DOESN'T SETTLE THE MECHANISM

HIGH YIELD
(Resolving Specific Forks)

- ✓ • Distinguishing Type 2B vs Platelet-type VWD.
- ✓ • Confirming Type 2N VWD.
- ✓ • Evaluating severe Type 3 VWD.
- ✓ • High-stakes family inheritance counseling.



LOW YIELD
(Mild Quantitative Reductions)

- ✗ • Evaluating mild Type 1 / Low VWF.
- ✗ • The VWF gene is highly polymorphic; many variants are not clearly pathogenic.

RULE: Phenotype remains the basis of classification. Genotype supports, clarifies, or challenges that classification. It does **not replace clinical judgment.**

DIAGNOSTIC TRAPS & GUARDRAILS

THE PREANALYTIC TRAP

Inflammation, pregnancy, exercise, acute bleeding, and poor sample handling (e.g., refrigerating whole blood) distort baselines. Fix the preanalytics before ordering specialized assays.

THE OVER-TESTING TRAP

The ‘abnormal value → order everything’ approach is a clinical error. Ordering multiple second-line tests without a clear diagnostic question generates a pile of poorly integrated data and ambiguous findings.

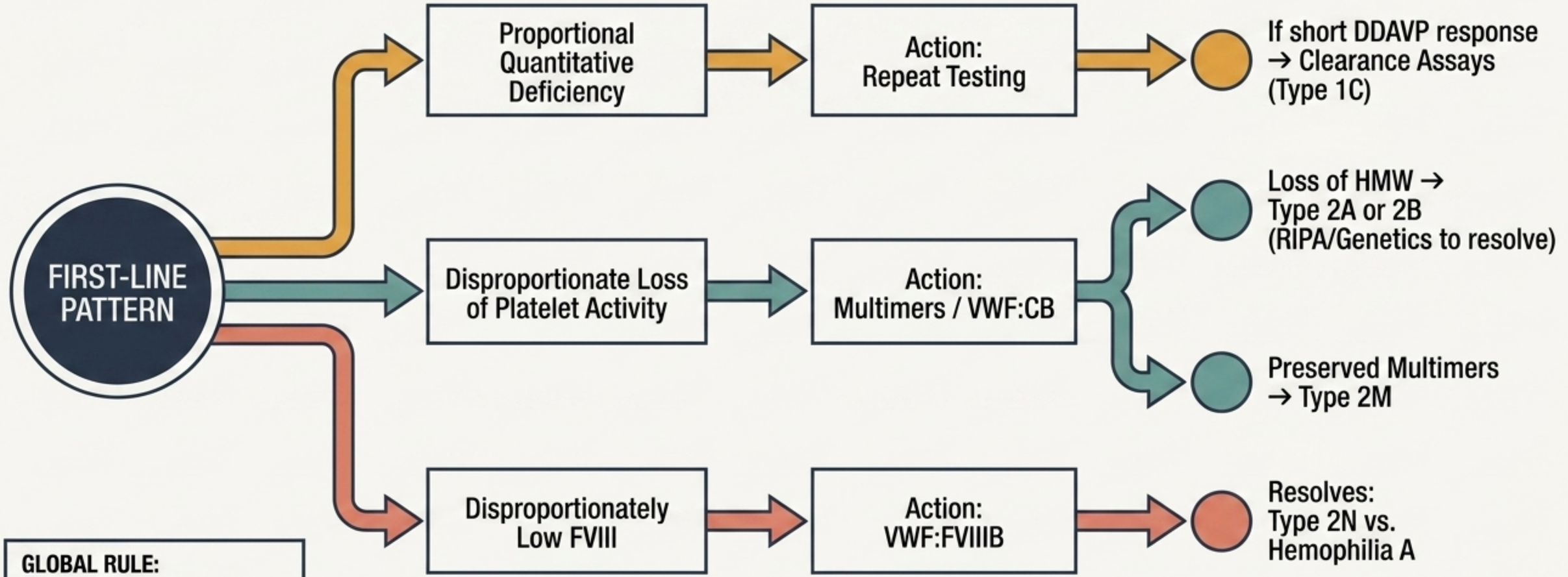
THE ACQUIRED VWS REMINDER

Older patients with no lifelong bleeding history or family history may have Acquired von Willebrand Syndrome (AVWS), which can perfectly mimic inherited Type 2 laboratory patterns.

THE BOUNDARY ADJUDICATION MATRIX

BOUNDARY (THE FORK)	KEY FIRST-LINE CLUE	DIFFERENTIATING SECOND-LINE TEST	CORE MECHANISM ADJUDICATED
Type 1 vs. Low VWF	Proportional reduction	Repeat testing / Genetics	Clinical significance of mild quantitative loss
Type 2A vs. Type 2B	Loss of HMW multimers	Low-dose RIPA / Genetics	Enhanced platelet binding (2B) vs absent (2A)
Type 2A vs. Type 2M	Low activity/Ag ratio	Multimer Analysis	Loss of architecture (2A) vs preserved (2M)
Type 2B vs. Platelet-Type	Enhanced RIPA response	Targeted Genetic Testing	Defect in VWF (2B) vs Platelet receptor (PT)
Type 2N vs. Hemophilia A	Disproportionately low FVIII	VWF:FVIIIB / Genetics	Defect in carriage (2N) vs primary deficiency (Hem A)

SYNTHESIS: THE LOCALIZATION MASTER MAP



GLOBAL RULE:
If the pattern is unstable or borderline, do not use advanced assays to force certainty prematurely. Repeat first-line testing.

JUDGMENT OVER TESTING

Second-line testing cannot:

- Substitute for a thorough bleeding history.
- Determine, by itself, if symptoms are clinically meaningful.
- Erase assay variability or convert continuous biology into clean categories.
- Decide whether treatment is needed.

Second-line tests localize mechanism. They do not replace clinical synthesis.
The goal is not to order more. The goal is to ask better.