KininX

ANGIOEDEMA DIAGNOSTIC
A GLOBAL SOLUTION

KininX is an expert in the field of angioedema diagnostics. This expertise is based on 20 years of fundamental and clinical research in the field of bradykinin angioedema (BK-AE) diagnostics. Today KininX proposes a unique global solution for BK-AE biological diagnosis based on ready-to-use innovative assays with online support guaranteed by our team of experts: Pr C. Drouet, Dr D. Ponard, Dr A. Ghannam and Dr D. Charignon.

1. Angioedema a complex disease of contact phase

Bradykinin angioedema is a rare disease characterised by sub-cutaneous and sub-mucosal oedema which could affected the face, the abdomen or the upper airways where it could be life-threatening (**Figure 1**). BK-AE is the consequence of bradykinin (BK) accumulation on the vascular endothelium. BK is released following the contact phase activation (**Figure 2**). Contact phase is under the powerful control of C1 Inhibitor (C1Inh).

BK-AE could be due to: (1) a hereditary or acquired C1Inh deficiency, (2) an increased BK production that is C1Inh independent *eg. mutation of* FXII or (3) a catabolism enzyme defect *eg.* iatrogenic angioedema induced by an anti-hypertension ACE inhibitor (**Figure 3**).

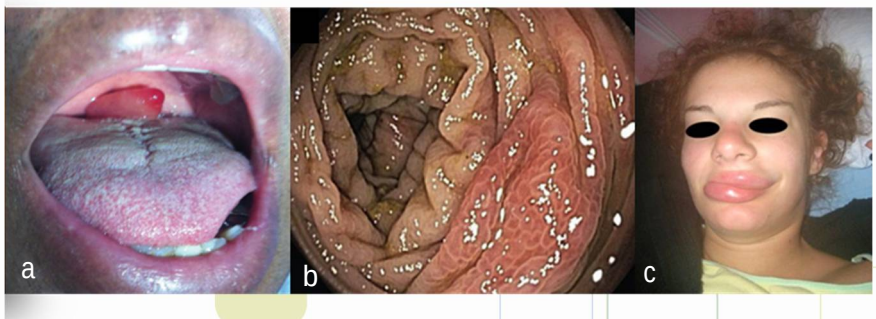

Figure 1 : Angioedema attack illustrations. *(a) Isolated Uvular Angioedema (Viana-Tejedor and Nunez-Gil, 2014); (b) Digestive mucosal Wong JCT2013; (c) Facial edema (Dr Bouvier M).*

2. Lack of BK-AE objective diagnostic criteria

BK-AE is a rare disease with **a difficult diagnosis.** It is often confused as the much more common histaminic angioedema. Today the diagnosis of BK-AE is based only on the physician's experience using clinical and therapeutical arguments. The biological diagnosis is exclusively based on the C1Inh investigations.

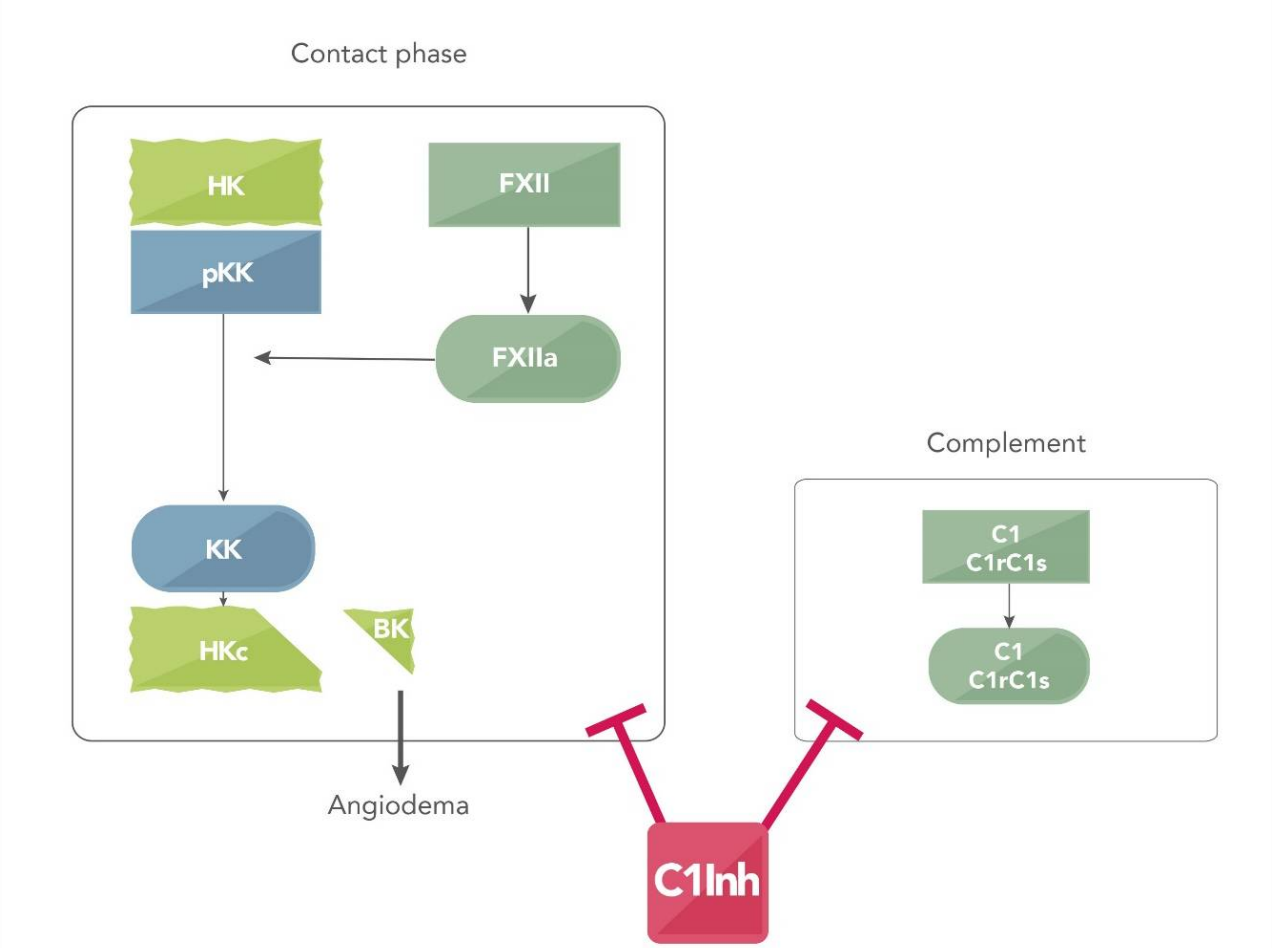

Figure 2 : C1 Inhibitor control contact phase and complement. *Factor XII (FXII) activates into FXIIa. FXIIa then activates prekallikrein (pKK) into kallikrein (KK) which then cleaves high molecular weight kininogen (HK) to release Bradykinin (BK). FXIIa and KK are under the powerful control of C1 Inhibitor (C1Inh). C1Inhibitor also control the complement and C1s protease.*

3. Current biological diagnosis tools based on complement are not efficient

Common BK-AE biological diagnosis targets complement as C1Inh antigen, C1Inh function using C1s protease as target, C1q antigen, C4 antigen and autoantibodies against C1Inh. These assays display low sensitivity as only 20% of BK-AE patients are properly diagnosed (Dessart et al., 2015). As consequence 80% of patients do not have an accurate diagnosis for years after the manifestation of their first symptoms (Aygören-Pürsün et al., 2014).

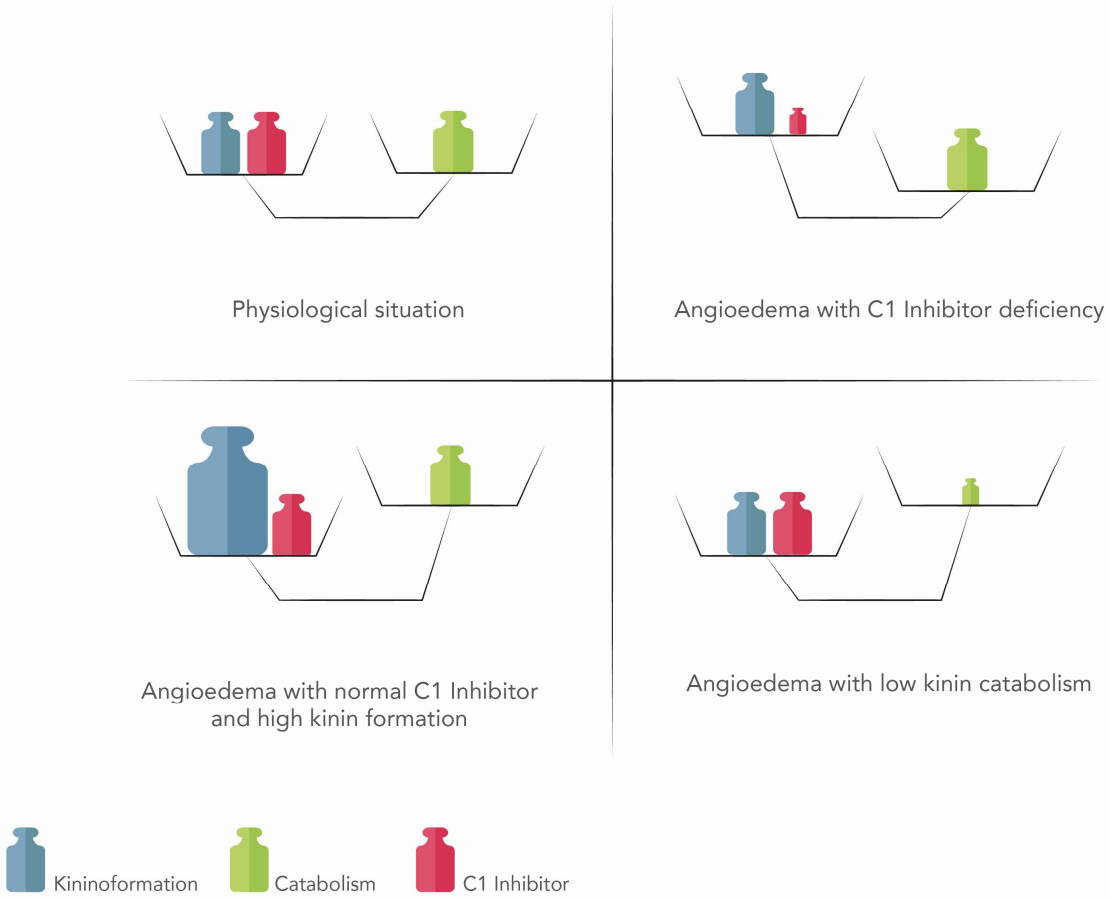

Figure 3: Angioedema causes. *Bradykinin metabolism could be disrupt for many reasons, a lack of control, an increased production, a decreased catabolism.*

4. New generation tools for angioedema diagnosis

KininX develops innovative assays to improve the diagnosis of BK-AE using biological markers. Our new generation of biological tests allows for a more effective biological diagnostic for more than 80% of patients.

What approach does KininX propose for the accurate diagnosis of BK-AE? (Figure 4)

The first test that is normally carried out is for C1inh function which is normally measured by targeting a complement protease, C1s. This is not ideal and for more accurate diagnosis it is best to transition towards assays targeting contact phase. As illustrated in Figure 2, C1Inh function targeting KK, displays higher sensitivity than the method targeting C1s protease. In the case of C1Inh deficiency, investigations can continue by sequencing the *SERPING1* or testing for autoantibodies against C1Inh in a clinical context. Nevertheless C1Inh only accounts for around

20% of BK-AE. For the other 80% of patients with normal C1Inh, an additionnal line of diagnostic tools must be followed.

The second biological line of testing is for kininogenase activity (Defendi et al., 2013) This includes two parameters: spontaneous amidase activity and proenzyme reserve. Spontaneous amidase activity is increased constantly in the case of C1Inh deficiency or transiently during an angioedema attack in case of normal C1Inh. Proenzyme reserve is consumed in case of chronic contact phase activation and during an acute attack.

Kininogenase activity, if measured during an attack, is found to be disrupted in 78% of BK-AE (20 % with C1Inh deficiency and 58 % with normal C1Inh) (Dessart et al., 2015). Investigations could be completed with the research of *F12* gene variants. However these variants are present in only about 4% of BK-AE patients.

The third biological line of investigation is kinin catabolism. If C1Inh function and kininogenase activity were normal but the clinical phenotype is suspect for BK-AE; diagnosis could be continued with the investigation of kinin catabolism. Kinin catabolism could be decreased in some rare hereditary deficiency (Dessart et al., 2015; Mathews et al., 1986) or in frequent iatrogenic angioedema (Cilia La Corte et al., 2011). Drugs which affect kinin catabolism such as anti-hypertensive drugs *eg*. angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor antagonist (sartan) and anti-diabetic drugs as dipeptidyl peptidase IV inhibitor (gliptine) could have BK-AE as side effect (Bas et al., 2015); such drugs affect kinin catabolism. Kinin catabolism investigation is also useful to understand a patient phenotype severity, because a decrease of kinin catabolism enzyme activity is associated with severe disease (Charignon et al., 2014; Drouet et al., 2008).

For all BK-AE patients, a family investigation should be considered in order to have effective information about the disease.

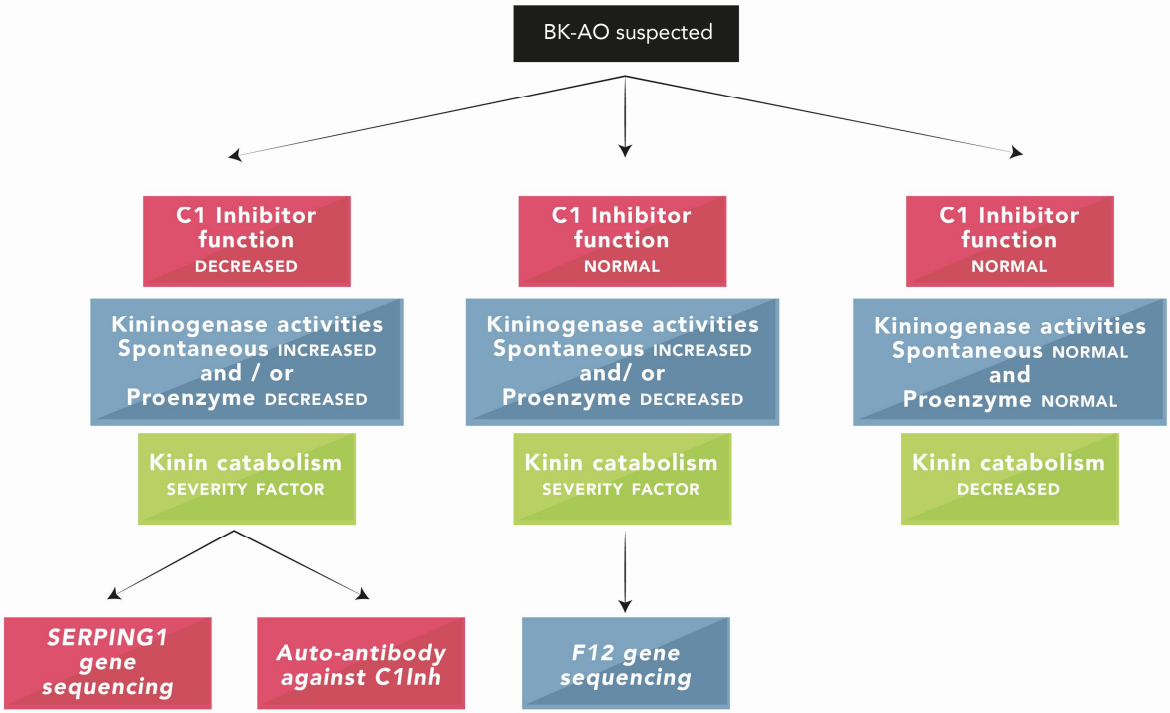

Figure 4: The biological approach preconize by KininX in front a BK-AE suspicion

What solutions KininX develops to realize this approach?

KininX develops a **global solution for BK-AE biological diagnostic,** composed of innovative assays and an online support with KininX's experts (Figure 5).

The first kit is a new method developed by KininX to measure C1Inh function using KK as target (Ghannam et al., 2015). It is based on the AE physiopathology and resulted of the contact phase activation. A comparison of 409 samples for C1Inh function using both methods targeting C1s or KK, demonstrate a 100% specificity for each method and a better sensitivity for KK target 98% than for C1s target 84% (Charignon et al., 2017a).

The second kit examines kininogenase activity, and reflects the ability to cleave HK to release BK (Defendi et al., 2013). Kininogenase activity is divided in two parts.

The first, the **spontaneous activity**, represents the systemic activity at the time of the sample. Consequently, this activity varies with the disease activity at the time of sample collection. For example, spontaneous amidase activity is increased during an angioedema attack with normal C1Inh function and is normalized 48h after the attack. From 185 with inherited C1Inh deficiency BK-AE patients, two parallel parameters are compared. Antigenic C4 with 71% sensitivity and

100% specificity and spontaneous kininogenase activity which presents higher performance values 97% sensitivity and 96% specificity (Charignon et al., 2017b).

The second, the **proenzyme activity** is the plasmatic capacity to be activated. Proenzyme stock decreased after contact phase acute or chronic activation.

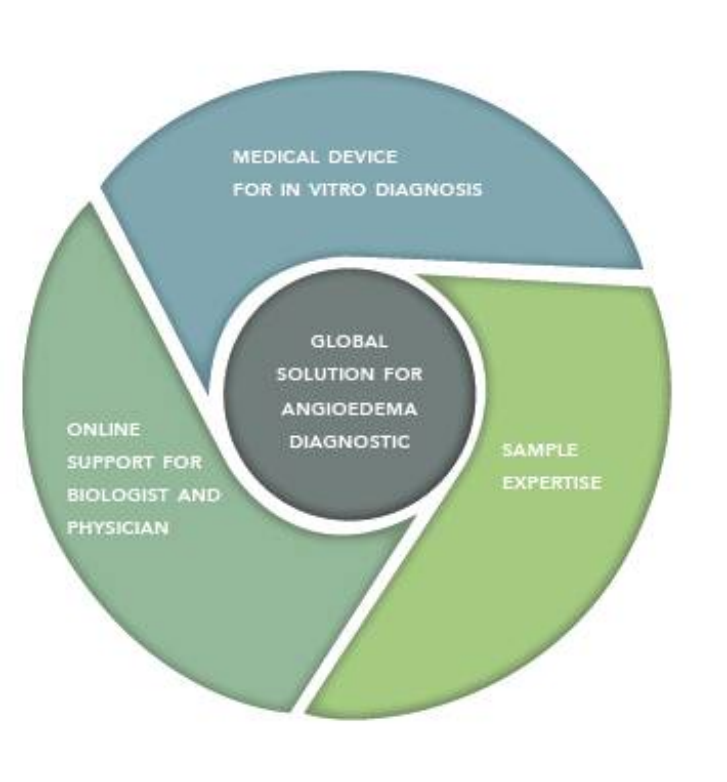

Figure 5: KininX global and innovative solution for angioedema diagnosis

These assays allow a proper diagnosis for 78% of BK-AE patients (Dessart et al., 2015), for the other 22% of patients, KininX develops a series of assays in regard to their clinical situation including kinin catabolism (Aminopeptidase P, Dipeptidyl Peptidase, Angiotensine-I converting enzyme and Carboxypeptidase N activities) and kininogen cleavage.

Finally, KininX makes available its expertise on angioedema biological diagnosis for physicians and biologists with an online support platform to help with data interpretation.

Dr D. Ponard (C1Inh expert at Grenoble Hospital) "Biologist had, during a long time, only the C1Inh protein exploration to diagnose BK-AE. It is exciting to have additional tools. This allows the clinician to strengthen his questioning with regard to the clinical observation. He can then adopt the adequate therapeutics."

5. Improve disease management

Thanks to the KininX solution, medical labs have a turnkey and low price solution for biological BK-AE diagnostics. Physicians and biologists have access to expert support for data interpretation. With the KininX biology based solution the proportion of BK-AE patient diagnosed is increased from 20% up to 78%. Using more pertinent innovative methods for angioedema disease, our ambition is to improve patient care.

6. References

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