CAT
Critically Appraised Topic

Titel: Contribution of molecular diagnosis in eosinophilia/hypereosinophilia

Eosinophilia
Hypereosinophilia
Hypereosinophilic syndrome

Immune mediated hypereosinophilia
Chronic eosinophilic leukemia (NOS)/ Idiopathic hypereosinophilic syndrome
Myeloid and lymphoid neoplasms with abnormalities of *PDGFRA, PDGFRB, FGFR1*

Author: Laetitia Brassinne
Supervisor: Prof Dr P Vandenberghe, Dr C Brusselmans
Date: 15/05/2014

CLINICAL BOTTOM LINE

Hypereosinophilic syndrome (HES) is a rare chronic disorder characterized by overproduction of eosinophils in the bone marrow, which results in marked and sustained peripheral blood eosinophilia. Sometimes, this leads to eosinophilic infiltration and functional damage of peripheral organs (1). It is often difficult to make the differential diagnosis between the diverse causes of hypereosinophilia. However, correct characterization of eosinophilia is important because treatment is dependent on the underlying etiology (16).
Since *Chusid et al.* described the first diagnostic criteria for "hypereosinophilic syndrome" in 1975 (2), a lot of research was done and helped to better understand this complex pathology. In 2002, *Gleich et al.* discovered the efficacy of imatinib mesilate for the treatment of hypereosinophilic syndrome (3). One year later, *Cools et al.* discovered an interstitial deletion in chromosome 4 leading to the formation of a novel gene by fusion of the Platelet-Derived growth factor alpha (*PDGFA*) and *FIP1-like-1 (FIP1-L1)* genes, coding for a tyrosine kinase that can be inhibited by imatinib in idiopathic HES (4,7). The number of cases however is small and currently we don't really know what are the long-term results of treatment with imatinib (7).
In UZ-Leuven, we observed that since the discovery of this rearrangement *FIP1L1-PDGFRA*, this molecular analysis is frequently requested in the context of a hypereosinophilia. This test is in most cases negative. In fact we observed in the current retrospective study that, between 2004 en 2013, from the 157 patients that were tested in UZ Leuven, only 2 patients were found positive (1,3 %).
In the current study we tried to develop a scoring system including clinical symptoms and laboratory tests in order to establish the necessity of performing this molecular test. The implementation of a scoring system seems to be necessary in UZ Leuven for clinical, organizational/logistic, economic and strategic reasons.

CLINICAL/DIAGNOSTIC SCENARIO

Eosinophils are highly specialized granulocytic effector cells that produce and store diverse biologically active molecules (cytotoxic, cytostimulatory proteins, lipid mediators, chemotactic peptides and cytokines). The most potent growth factors for eosinophils are IL-5, IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF). Under various conditions, eosinophils can invade certain target organs and secrete their products into the surrounding tissue, thereby triggering local inflammation and tissue remodeling. The contents of eosinophil granules may also promote hypercoagulability, enhancing the thromboembolic risk. The normal eosinophil count in peripheral blood ranges from 0.05 to 0.5 x 10^9/L en normal values of eosinophils in bone marrow (BM) aspirates commonly range between 1% and 6%. We can divide blood eosinophilia into mild (0.5-1.5 x 10^9/L), marked (>1.5x10^9/L) and massive (>5.0 x 10^9/L) eosinophilia. The term hypereosinophilia (HE) should be used when tissue and/or blood eosinophilia is marked and persistent. *P Valent et al.* agreed that the term persistent applies to peripheral blood eosinophilia recorded on at least 2 occasions with a minimum time interval of 4 weeks, except when immediate treatment is necessary because of HE-related organ dysfunction. Blood and/or tissue HE is detectable in various inflammatory reactions, sometimes in patients with solid tumors and certain hematologic malignancies. Based on underlying conditions and etiology, several variant forms of HE exist for which recently a terminology has been proposed (Table 1. Classification of the major causes of hypereosinophilia) (5, 6, 16, 18).

Table 1. Classification of the major causes of hypereosinophilia (HE)

- Secondary/reactive HE (nonclonal cells) : **HE_R**
  - ✓ Helminth infections
  - ✓ Drug reactions (allergic or toxic)
  - ✓ Other allergic reactions
  - ✓ Atopic diseases
  - ✓ Scabies, other infestations
  - ✓ Allergic bronchopulmonary aspergillosis
  - ✓ Chronic inflammatory disorders (eg, IBD)
  - ✓ Autoimmune diseases (eg, skin diseases)
  - ✓ Connective tissue diseases
  - ✓ Metabolic abnormalities
  - ✓ Solid tumors/malignancy
  - ✓ Chronic graft-versus-host disease
  - ✓ Hodgkin disease
  - ✓ B- or T-cell lymphoma/leukemia
  - ✓ Langerhans cell histiocytosis
  - ✓ Indolent systemic mastocytosis
- Hereditary/familial form : **HE_{FA}**
- Clonal (neoplastic) hypereosinophilia : **HE_N**
  - ✓ Chronic eosinophilic leukemia – Not Otherwise Specified (NOS)
  - ✓ Hematopoietic neoplasms with eosinophilia and abnormalities in *PDGFRA*
  - ✓ Hematopoietic neoplasms with eosinophilia and abnormalities in *PDGFRB*
  - ✓ Hematopoietic neoplasms with eosinophilia and abnormalities in *PGFR1*
  - ✓ CML with eosinophilia (CML-eo)
  - ✓ AML with inv(16) and eosinophilia (AML-M4-eo)
  - ✓ *JAK2 V617F*$^+$ MPN with eosinophilia (MPN-eo)
  - ✓ ASM with eosinophilia (ASM : Aggressive systemic mastocytosis)
  - ✓ MDS with eosinophilia (MDS-eo)
  - ✓ MPN/MDS overlap syndromes with eosinophilia
- HE of undetermined significance : **HE_{US}**

In this report, we will only review patients in whom HE remains unexplained after a detailed medical history and after testing in order to exclude secondary/reactive HE and hereditary HE. In patients with unexplained HE, there is frequently the need for invasive and costly analysis, including bone marrow (BM) cytology, histology and immunochemistry, cytogenetics, molecular analyses (10, 16).
In patients with myeloid or stem cell-derived neoplasms, eosinophils usually belong to the malignant clone. In some cases, fusion genes involving *PDGFRA* may be present. The *FIP1L1-PDGFRA* fusion gene (and the related cytogenetic surrogate CHIC2 deletion by interphase fluorescence in situ hybridation) is detected in approximately 10-20% of all cases, and thus is the most frequent fusion oncoprotein and target detectable in patients with this pathology. *Baccarani et al.* observed that he frequency of the *FIP1L1-PDGFRA* fusion appears to be low and also more frequent in men than in women (7). Furthermore clinical manifestations are splenomegaly and/or hepatomegaly, serum elevated B12, serum elevated tryptase, cardiac infiltration, fatigue. Some case series have shown that elevated B12 and tryptase have a strong correlation with the *FIP1L1-PDGFRA* translocation (13, 14, 22).

The detection of the *FIP1L1-PDGFRA* fusion has a great impact on the treatment of HES/*FIP1L1-PDGFRA* positive (HES F/P+), because several clinical trials demonstrated that when this mutation is present, imatinib can be used as first line therapy. The United States Food and Drug Administration (FDA) approved imatinib for patients with HES F/P+ in October 2006 (3,7,17,18,19,20,21). This fusion gene product seems to be also sensitive to other tyrosine kinase inhibitors (TKI) such as nilotinib or dasatinib. But nilotinib or dasatinib are usually second line therapy in case patients develop intolerance or resistance to imatinib which is often due to the emergence of clones expressing mutant forms of *PDGFRA* that are less sensitive to imatinib inhibition (11, 12). When patients were early treated with imatinib that prevents irreversible organ damage in most of them. Therefore, it is of great importance to early identify patients with a myeloid neoplasm likely to respond to imatinib therapy. The current recommendation is to treat even asymptomatic patients with HES/F/P+ with a low-dose imatinib mesilate (100mg/day) (4,5,6,15,24). Some studies demonstrated also hematologic benefit of imatinib in patients with CEL-NOS or HES without a demonstrable *FIP1L1-PDGFRA* mutation. Nevertheless, hematologic responses in this group are more often partial and short lived compared with those occurring in patients with HES/F/P+. For those patients also a higher-dose imatinib is required (8,9,15,21).

QUESTION(S)

1) It seems to be interesting to do a study of RT-PCR and FISH currently used in UZ-Leuven for the diagnostic of a HE *FIP1L1-PDGFRA* (+) : Why only 2 patients were found positive (1,3 %) in 9 years?
2) What are the benefits of a scoring system to establish the necessity to carry out a molecular analysis (detection of the rearrangement *FIP1L1-PDGFRA*) in the case of a hypereosinophilia?

SEARCH TERMS

1) MeSH Database (PubMed): MeSH term : "eosinophils", "hypereosinophilia", "hypereosinophilic syndrome", "chronic eosinophilic leukemie", "eosinophilia and *FIP1L1-PDGFRA*", "hypereosinophilia and tryptase", "hypereosinophilia and vitamin B12"
2) www.google.com : search term: "hypereosinophilia", "hypereosinophilic syndrome" "chronic eosinophilic leukemia"

RELEVANT EVIDENCE/REFERENCES

1) Original Articles

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16. Montgomery ND et al. Diagnostic complexities of eosinophilia. Arch Pathol Lab Med 2013 – Vol 137
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21. Jain N et al. Imatinib has limited therapeutic activity for hypereosinophilic syndrome patients with unknown or negative PDGFRA mutation status. Leukemia Research 2009; 33 : 837-839.
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2) Reference Works, Handbooks and Databases :

WHO Classification of tumours of haematopoietic and lymphoid tissues (WHO 2008 4th Edition)

Introduction

In the literature, HES F/P+ is described as more commonly seen in men. This pathology may also be associated with palpable splenomegaly and/or hepatomegaly, cardiac damage, thrombus formation, markedly elevated white blood cell (WBC) count, markedly increased serum vitamin B12, elevated serum tryptase, normal immunoglobulin E (IgE) level. Besides, bone marrow biopsies are in some cases hypercellular and revealed a distinctively myeloproliferative aspect, the granules in chronic eosinophilic leukemia associated with *FIP1L1-PGFRA* are commonly small and sparse unlike in AML M4Eo, where granules are enlarged. It is however difficult to use abnormal eosinophil morphology (eg. Vacuolization or cytoplasmic hypogranularity, abnormal lobation, ring nuclei) to reliably distinguish reactive from clonal eosinophilia, because these cytologic changes may be present in both conditions. HES F/P+ patients with have an apparently normal karyotype. Those patients respond also poorly to steroids, hydroxyurea or interferon α (1,14,15,16,18, 22,23,24).

We noticed that since the detection of *FIP1L1-PDGFRA* mutation by Real-time Polymerase Chain Reaction (RT-PCR) and since Fluorescence in situ hybridization (FISH) is available in UZ-Leuven, 157 molecular tests were requested between 2004 and 2013 for the diagnosis of an hypereosinophilic syndrome.

With a retrospective study between 2004 and 2013, we will try to develop a scoring system, including clinical symptoms and laboratory tests, in order to establish the necessity of performing this costly molecular test when an hypereosinophilia is detected.

Patients, materials, and methods

Patients' selection

We conducted a single-institution retrospective study of 157 patients tested for the *rearrangement FIP1L1-PDGFRA* by RT-PCR en FISH between 2004 en 2013. We identified the patients by querying the UZ-Leuven electronic database. We found **2 patients that were *FIP1L1-PDGFRA* (+).** Besides, we analyzed 40 patients that were *FIP1L1-PGFRA* (-). Adult patients from whom we have all clinical and laboratories results that we need for our table and "scoring system" were included in the study. **18 *FIP1L1-PDGFRA* (-) patients** were selected and considered as negative controls (Table 2).

Real-time PCR (RT-PCR) in UZ-Leuven

The *FIP1L1-PDGFRA* rearrangement is analyzed by a reverse transcriptase-polymerase chain reaction (RT-PCR). RNA was extracted from EDTA-treated blood samples or bone marrow. (1)

Fluorescence in situ hybridization in UZ-Leuven

FISH analysis was performed on cytogenetic specimens of bone marrow. (1)

Determination of serum vitamin B12 in UZ-Leuven

Vitamin B12 also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is normally involved in the metabolism of every cell of the human body, especially affecting DNA synthesis and regulation, but also fatty acid synthesis and energy production.
Normal levels of vitamin B12 in serum are 191-663 ng/L.

In UZ-Leuven we use an immunoassay – competitive – electrochemic luminescence on the Hitachi (Roche) – Modular E. For this analysis, 1 mL of serum. This test is carried out every day.

Determination of serum tryptase in UZ-Leuven

Tryptases are proteases located in the secretory granules of human mast cells, and in small quantities also in basophils. There are several clinical conditions that are accompanied by an increased tryptase.
During anaphylaxis, mast cell granules release tryptase. In this case, tryptase is transiently elevated with peak levels between 15-120 minutes after the onset of the reaction. Afterwards, the tryptase level declines slowly within the next 3-6 hours. A return to baseline level can generally be verified after 24 hours.
By comparison, histamine (another immunologic mediator released by activated mast cells) is cleared from blood within minutes.
Increased serum levels may also occur after allergen challenge or in patients with systemic mastocytosis or mast cell activation syndrome. Raised tryptase to more than 20 ng/ml has been found in 30% of patients with acute

myeloblastic syndromes and in myeloplastic syndromes. Raised tryptase has also been described in a group of idiopathic hypereosinophilic syndromes.

In UZ-Leuven we use a Fluorescence Enzyme Immunoassay (FEIA), which is the only available commercial technique for the quantitation of tryptase. The assay is carried out, twice a week, on the ImmunoCap 1000 platform. Per test 0.5 mL of serum is needed. The measuring range is situated between 1-200 µg/L. Levels of total tryptase in serum, determined in healthy individuals, gave a geometric mean of 3.8 µg/L, with a 95 upper percentile of 11.4 µg/L, which is used as normal limit.

Determination of Total serum IgE in UZ-Leuven

IgE antibodies appear as a result of sensitization to allergens, and the measurement of circulating total IgE assists the clinical diagnosis of IgE-mediated allergic disorders.
Normal levels of total IgE in serum of adults are ≤ 114 kU/L (≥ 18 years).

Total serum IgE in UZ-Leuven is also a Fluorescence Enzyme Immunoassay (FEIA) carried out on the ImmunoCap 1000 analyzer. Per test 1 mL of serum is required. The test is carried out every weekday.

Results

The *FIP1L1-PDGFRA* fusion transcript was detectable in 2 out of 157 (1,3%) patients for which this test was carried out in the period between 2004 and 2013, in our institution. Both patients were males. The first patient was 78 years old at the time of diagnosis in 2005 and the second was 74 years at diagnosis in 2011. Their white blood cell (WBC) count was respectively 11,11 x 10^9/L and 15,97 x 10^9/L, with an absolute eosinophil count of 6,1 x 10^9/L and 8,7 x 10^9/L and eosinophilic bone marrow infiltration of 26% and 15,7%. No blast excess was detected. Vitamin B12 and tryptase were both increased. Serum IgE was within the normal range. The patients presented without organ involvements. Cytogenetic analysis revealed normal karyotypes. Both patients responded to imatinib at a starting dose of 100 mg daily.
We included also 18 patients as “negative controls”, in order to be able to compare the clinical symptoms and laboratory tests for establishing our scoring system. We decided to use non-invasive parameters for our scoring system. Therefore, we took an absolute eosinophil count > 5000/µl and >1500/µl, serum vitamin B12 level > 800 ng/L, serum tryptase level > 12 µg/L and total IgE < 20 kU/L. Using this scoring system, our two positive patients had a score of 6/6 or 5/5 without tryptase analyze. The negative patients (for the rearrangement *FIP1L1-PDGFRA*) had a score between 0-4. (Table 2. Score system)

Table 2. Score System

SCORE SYSTEM patient number	Absolute eosinophil count > 5000/µL	Absolute eosinophil count > 1500/µL	Serum Vit B12 level > 800ng/L	Serum tryptase level > 12 µg/L	total IgE < 20 kU/L	Som biochemische parameters (5)	TEST : YES 3-6 NO 0-2	SCORE SYSTEM without Tryptase	Absolute eosinophil count > 5000/µL	Absolute eosinophil count > 1500/µL	Serum Vit B12 level > 800ng/L	total IgE < 20 kU/L	Som biochemische parameters (4) without TRYPTASE	TEST : YES 3-5 NO 0-2
1	2	1	1	1	1	6	yes		2	1	1	1	5	yes
2	2	1	1	1	1	6	yes		2	1	1	1	5	yes
3	2	1	1	0	0	4	yes		2	1	1	0	4	yes
4	0	1	0	0	0	1	no		0	1	0	0	1	no
5	2	1	0	0	0	3	yes		2	1	0	0	3	yes
6	0	0	1	0	0	1	no		0	0	1	0	1	no
7	0	0	0	0	0	0	no		0	0	0	0	0	no
8	2	1	1	0	0	4	yes		2	1	1	0	4	yes
9	2	1	0	0	0	3	yes		2	1	0	0	3	yes
10	2	1	0	1	0	4	yes		2	1	0	0	3	yes
11	0	0	0	0	1	1	no		0	0	0	1	1	no
12	2	1	1	0	0	4	yes		2	1	1	0	4	yes
13	2	1	0	0	0	3	yes		2	1	0	0	3	yes
14	2	1	0	0	0	3	yes		2	1	0	0	3	yes
15	0	1	0	0	0	1	no		0	1	0	0	1	no
16	0	0	0	0	0	0	no		0	0	0	0	0	no
17	0	1	0	1	0	2	no		0	1	0	0	1	no
18	0	1	0	0	0	1	no		0	1	0	0	1	no
19	0	1	0	1	1	3	yes		0	1	0	1	2	no
20	0	1	1	1	1	4	yes		0	1	1	1	3	yes
	Conclusion : 12/20 useful test = 60 %								Conclusion: 11/20 useful test = 55 %					

Discussion

HE F/P+ is a rare hematologic disorder and the frequency of *FIP1L1-PDGFRA* fusion transcript in HE differs among published studies. It seems to be more frequent in patients corresponding to the "HES criteria" (even if there is no "real" consensus on the criteria for a HES diagnosis) and occurs in 14-17% of patients. This rearrangement was less frequently found when the unselected population of patients with prolonged hypereosinophilia was examined and varied between 4 and 10% (23). In our restrospective study, the *FIP1L1-PDGFRA* transcript was only found in 1,3% of patients, which was considerably less than the frequency obtained in other studies.
The main objective of our study was to develop a non-invasive scoring system including clinical symptoms and/or laboratory tests. We choice to use 4 laboratory tests, because we observed that clinical signs did not differ between F/P+ and F/P- patients. Indeed, we observed no splenomegaly, no hepatomegaly, no endomyocardial pathology, and concerning thrombus formation there was no increase in both groups. In the literature, HE with *FIP1L1-PDGFRA* mutation was frequently associated with increased vitamin B12, increased tryptase levels and normal IgE values. Therefore, we decided to use for our scoring system : absolute eosinophil count > 5000/µl and > 1500/µl, serum vitamin B12 level > 800 ng/L, serum tryptase level > 12 µg/L and total IgE <20 kU/L. This resulted for our 2 positive patients in a score of 6/6.
Using this scoring system, even if we do the analysis when the score is ≥ 3/6, we will reduce the molecular analysis for *FIP1L1-PDGFRA* rearrangement significantly, namely by 40-45%.
Unfortunately, it is difficult to validate this scoring system due to the lack of positive patients in UZ-Leuven.

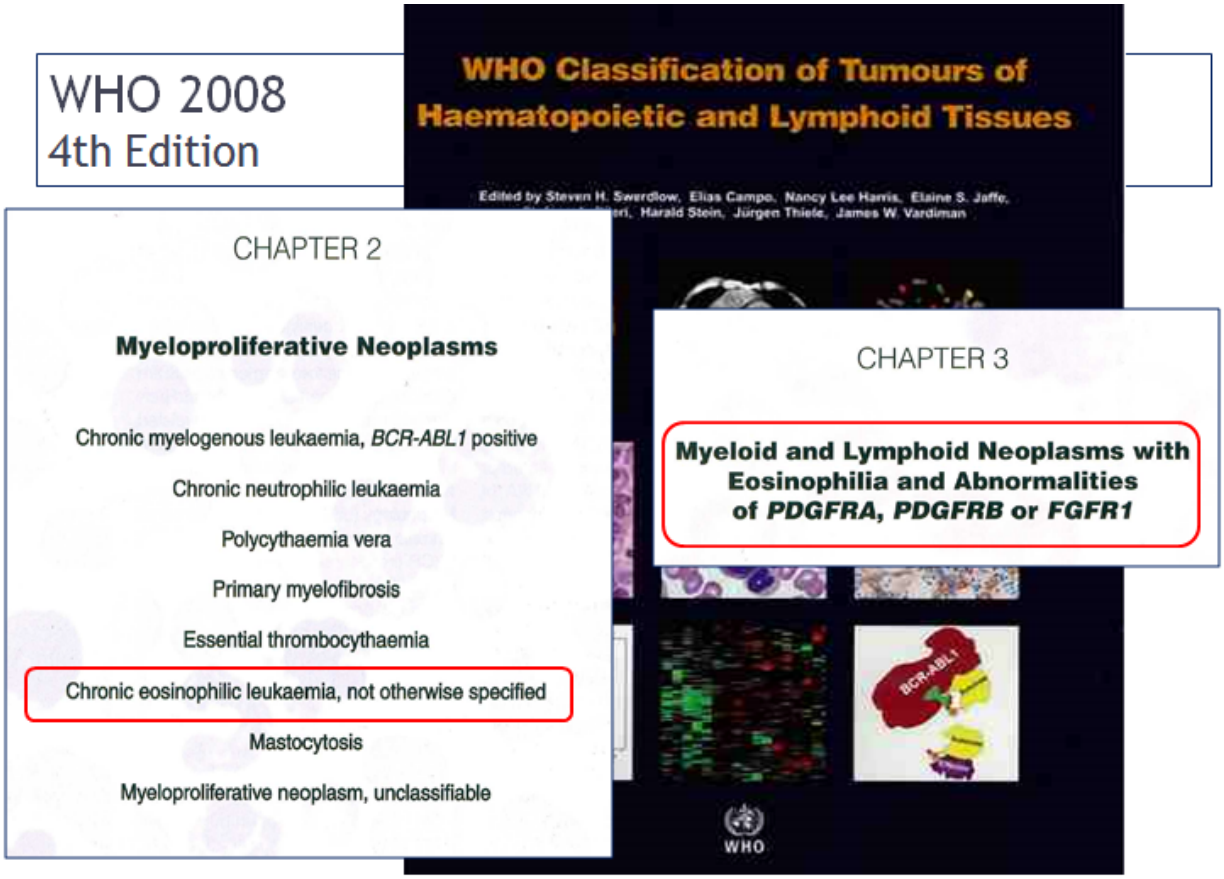
TO DO/ACTIONS

1) Survey of testing in other centers? (3 other centers in Belgium)

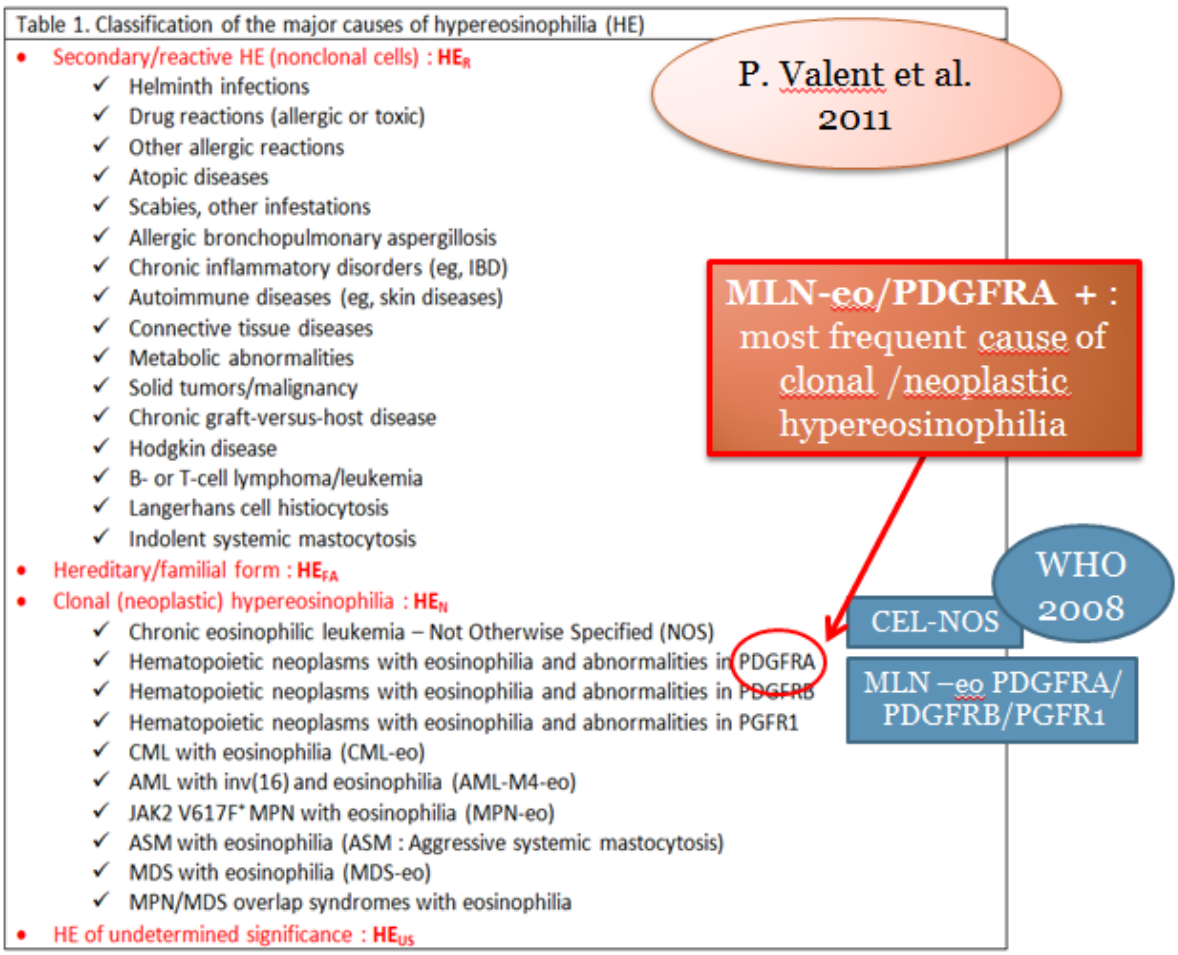
2) Discuss need for validation of scoring system as means for selecting patients who need this analysis

ATTACHMENTS

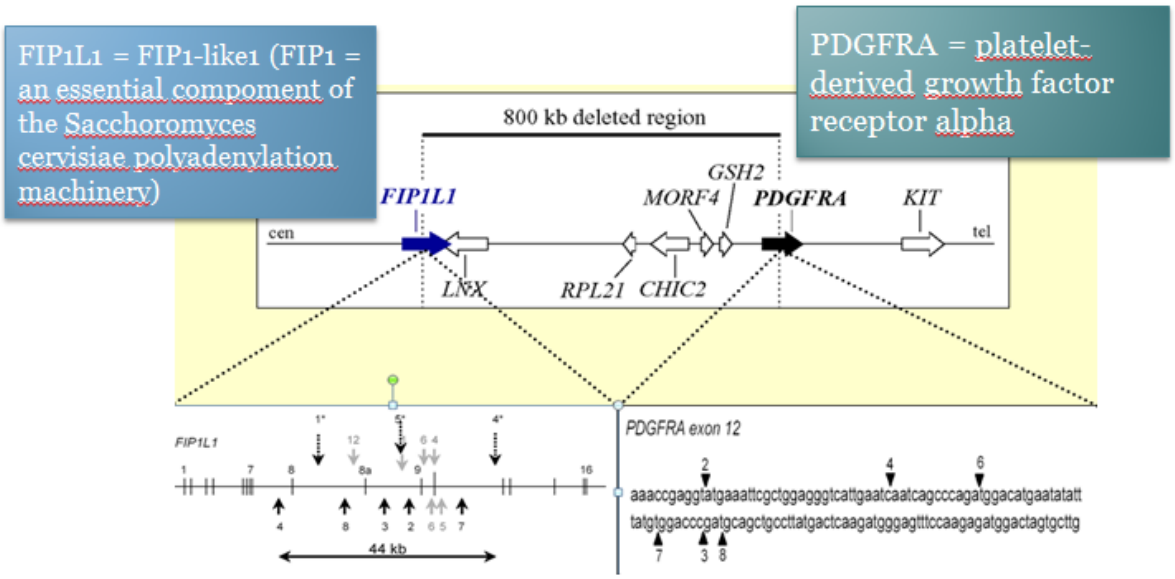
Attachment 1



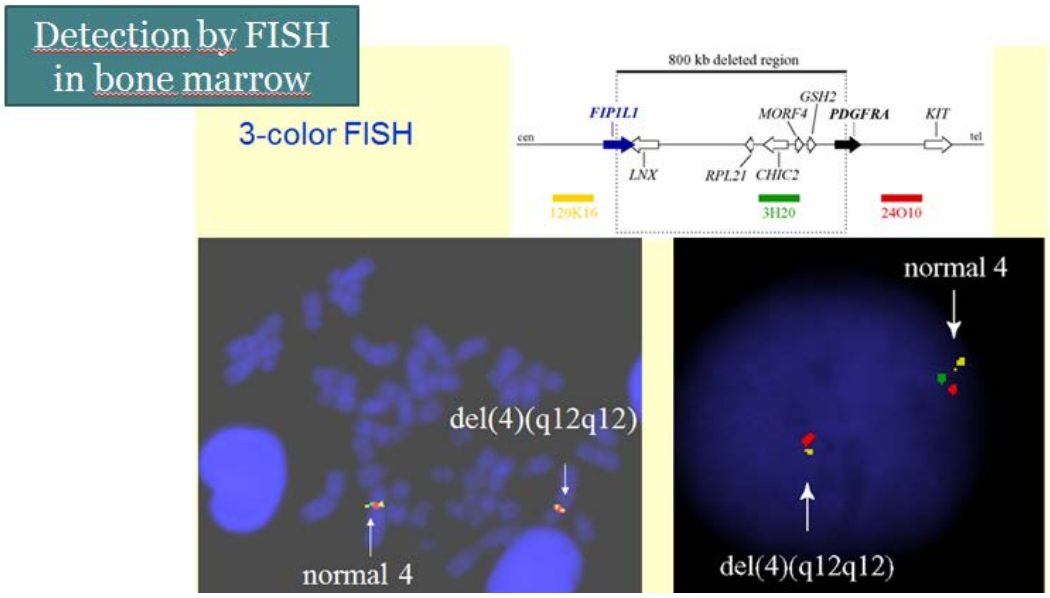
Attachment 2



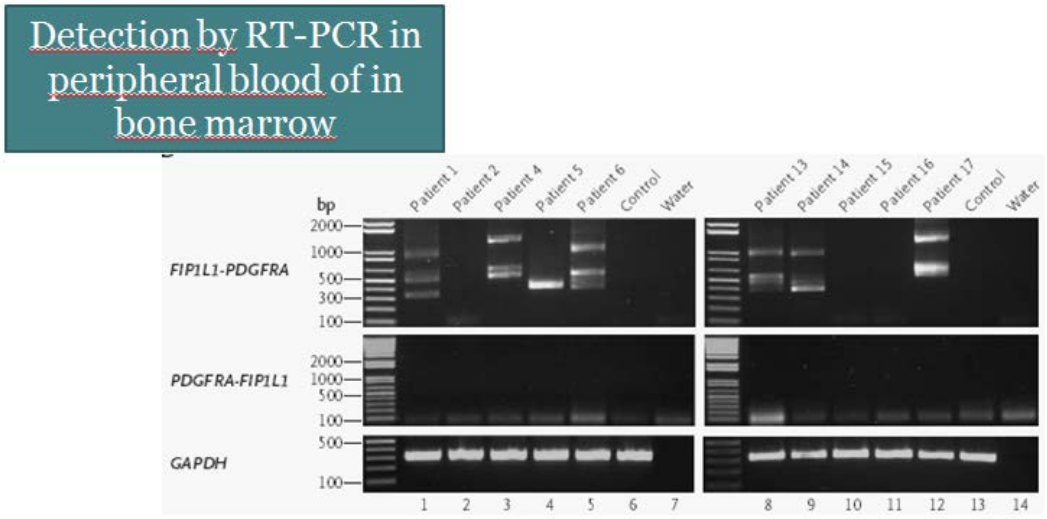
Attachment 3



Attachment 4



Attachment 5



Cools et al., NEJM, 2003, 348, 1201

Attachment 6

lab_name	test_name	contact	comments
AZ Sint-Jan AV Brugge	FIP1L1/PDGFRA qualitative analysis	Johan Billiet/Friedel Nollet	nested RT-PCR; primers from Cool-J et al. NEJM (2003)
CMG-UZGent	FIP1L1/PDGFRA qualitative analysis	nadine van roy	FISH analysis
CHU Liège - Hemato	FIP1L1/PDGFRA qualitative analysis	Dr Frédéric Lambert	As described by Cools et al., NEJM, 2003. Please, provide us with EDTA anticoagulated blood (10 ml) or bone marrow (3 ml) within 16H post-sampling OR cells within Trizol/Tripure solution OR preserved cells within RNALater solution.
UZ-KULeuven CEMOL	FIP1L1/PDGFRA qualitative analysis	Peter Vandenberghe / Els Lierman	Sample type: EDTA-blood / EDTA-bone marrow, minimal volume: 5ml Frequency: 2x/week Test type: nested PCR on cDNA, based on Cools et al, NEJM 2003.

http://www.moleculardiagnostics.be/