Ironing out the mechanism of anemia in celiac disease

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Celiac disease has been recognized as a distinct clinical entity since antiquity. The term *celiac affection* was first introduced by Aretaeus of Cappadocia, living in the second century,[1] and the first modern description of the disease was by a London pediatrician, Samuel Gee, in 1887. Although the causative agent of celiac disease was unknown, Gee correctly assumed that diet would offer a cure. Gee recognized that milk intolerance is a problem with celiac children and recommended that highly starched foods should be avoided. The link with wheat was not made until the 1940s by the Dutch pediatrician, Willem Dicke, and it is possible that the clinical improvement of patients during the 1940s when flour was scarse may have contributed to his discovery. The role of the gluten component of wheat was discovered by Anderson *et al.* in 1952 and villous atrophy shortly thereafter by Paulley in 1954 employing biopsies taken from patients during abdominal operations to avoid autolysis. The acquisition of fresh histological samples was greatly simplified following the description of the principles of small bowel biopsy in 1956 by Margo Shiner.

Pathogenesis

The key steps underlying the intestinal inflammatory response in celiac disease have been reviewed recently by van Heel and West.[2] The toxic fractions of wheat proteins relevant to celiac disease have now been well characterized. Because of their high proline and glutamine content, gluten peptides are hydrophobic and resistant to degradation by gastric, pancreatic and intestinal brush border membrane proteases. Several peptides of key importance have been identified. A-gliadin p31-43 induces interleukin 15, a key cytokine involved in T-cell activation (Figure 1). Interleukin 15 induces the expression of a stress molecule, MICA (major histocompatibility complex class I-related chain A) on enterocytes and upregulates NKG2D (activating natural killer cell receptors) on intraepithelial lymphocytes (IEL). This interaction results in direct enterocyte killing and is a likely cause of villous atrophy.[3]

The role of the human leukocyte antigen region (HLA) in celiac disease has been suggested by the observation that nearly all patients with celiac disease possess either the HLA-DQ2 (90%) or HLA-DQ8 heterodimer.[4] However, HLA-DQ2 is also found in 30% of the healthy Caucasian population indicating that it is a necessary but not sufficient component for the development of celiac disease. In practical terms, testing for HLA-DQ2 is useful for the exclusion of suspected celiac disease but is of limited usefulness for a positive diagnosis.

The participation of HLA-DQ2 in the pathogenesis of celiac disease is described schematically on the left side of Figure 1. In addition to the role of A-gliadin p31-43 described above, a second gluten peptide p57-73 is presented to mesenteric lymph node T cells by HLA-DQ2 on antigen presenting dendritic cells (DC). HLA-DQ2 preferentially binds peptides with negatively charged amino acids. Tissue transglutaminase (TTG), the target of antiendomysial antibodies in celiac disease, has an important role in converting glutamine to glutamate residues generating negatively charged amino acids that are better bound by HLA-DQ2. Although anti-TTG antibodies are highly specific and sensitive indicators of celiac disease, the primary role of such antibodies in the pathogenesis of this disease is unclear. Antigen specific mesenteric T cells subsequently migrate to the peripheral blood and home back to the intestine employing specific cell adhesion molecules, inducing cell death by cytokine release, mainly interferon-γ.

Diagnosis

Despite the availability of highly accurate serological tests, demonstration of histological changes of the small bowel mucosa is still regarded as the gold standard of diagnosis in celiac disease. These findings may range from subtle changes such as isolated increase in intraepithelial lymphocytes, to various degrees of villous atrophy, to total villous atrophy. Questionable cases should be reviewed by experienced pathologists who are familiar with the spectrum of mucosal changes in celiac disease. As the disease may be patchy, 4 to 6 biopsies should be obtained from the second part of the duodenum and the best results are achieved when the biopsies are *unfolded* and mounted with the villous side up to allow proper cross rather than tangential sectioning. As gluten reduction effects histological severity and serology, biopsies should be obtained before gluten withdrawal.

The discovery of antiendomysial antibodies in patients with celiac disease and dermatitis herpetiformis in 1984 had a major impact on the subsequent evolution of screening and diagnostic algorithms for diagnosing celiac disease.[5]

Antibodies to the endomysium, a connective tissue protein found in the collagenous matrix of the esophagus, are directed against tissue transglutaminase (TTG). IgA endomysial antibodies are measured in serum by indirect fluorescence using monkey esophagus or human umbilical cord as a substrate. Its specificity and sensitivity is estimated at 99% and over 90% respectively in both adults and children. Subsequently, an enzyme linked immunoabsorbent assay has been developed to measure anti-TTG IgA antibody activity. This test is more convenient, quantitative, less labor intensive, cheaper and yields comparable sensitivity and specificity to the older anti-endomysial indirect fluorescence test. Anti-gliadin antibody lacks accuracy and should no longer be performed.

IgA deficiency, the commonest human immunodeficiency, is 10-15 times more common in patients with

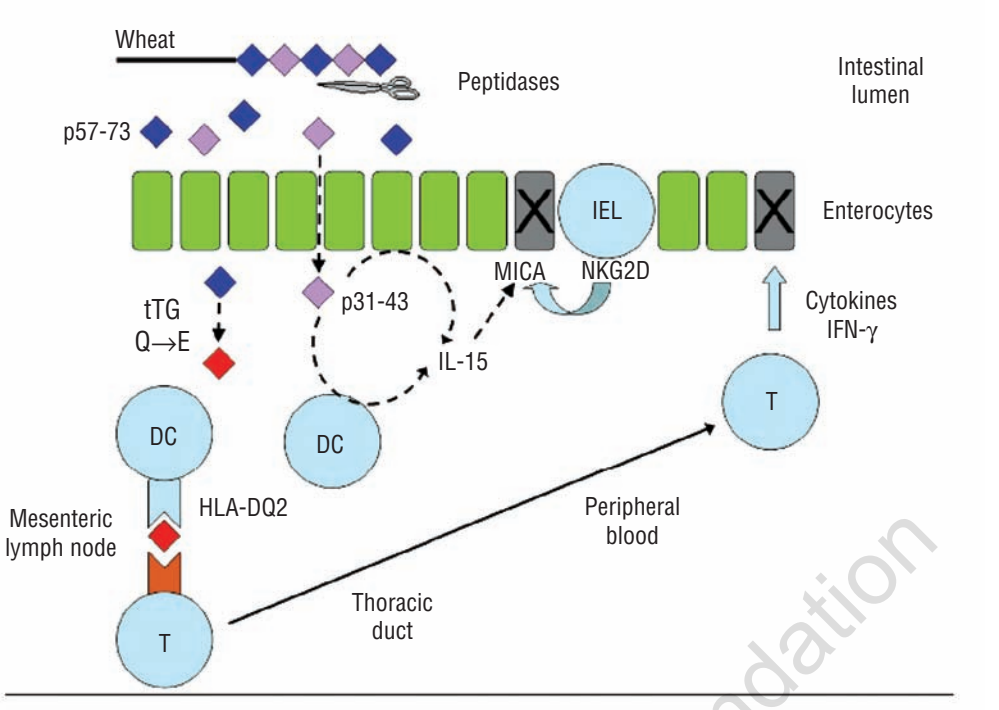

Figure 1. Pathogenetic mechanisms in celiac disease: key toxic sequences of gluten are resistant to intestinal proteases. Peptide p31-43 may directly induce IL-15 production and upregulate MICA (major histocompatibility complex class I-related chain A), a stress molecule on enterocytes. Another gluten peptide, p57-73, is deamidated by tissue transglutaminase (tTG) and presented to T cells by HLA-DQ2 on antigen presenting dendritic cells (DC). The initial triggering event occurs in the mesenteric lymph nodes. Thus, epithelial cytotoxicity occurs via at least two mechanisms: (i) cytokine release (especially interferon-γ) by antigen specific T cells, and (ii) directly by intraepithelial lymphocytes via MICA-NKG2D interaction (*Reproduced from van Heel and West*[2]*, permission pending*).

celiac disease with a prevalence that may reach 3%. As both anti-endomysial and anti-TTG antibodies are Iga based, IgA measurement should be performed as an integral part of antibody screening for celiac disease. Various algorithms have been proposed for the combined or consecutive use of serology and small bowel biopsy in screening for, or definitive diagnosis of celiac disease. According to one algorithm, a distinction should be made *a priori* between high-risk patients with clinical evidence of malabsorption syndrome, and low-risk patients with no such evidence. In all patients, initial screening for endomysial or TTG antibodies should be performed. In all high-risk patients, this should be followed by small bowel biopsy. However, in low-risk patients, negative serology should be regarded as evidence of a very low likelihood of missing celiac disease.[2]

Prevalence of celiac disease

The availability of simple and reliable serological tests for screening large numbers of subjects has allowed the study of celiac disease prevalence in the general population. The review of van Heel and West lists some of the most extensive recent studies involving between 1,000 to over 7,000 subjects each.[2] Despite some variations in methodology, mostly involving initial serological screening followed by confirmation by small bowel biopsies, the results were remarkably similar. In both children and adults, the prevalence of celiac disease was about 1%, although in some studies it was as low as 0.18 or as high as 5.66%. Interestingly, disease prevalence did not increase with age, implying that the phenotype of celiac disease is acquired at a very early age. The reason why at a certain point in time the symptomatology may be aggravated permitting clinical diagnosis is at present unknown. In contrast to the 1% prevalence of celiac disease diagnosed in prospective general population studies, it is estimated that the rate of clinically diagnosed cases is only 0.05 to 0.27%. Accordingly, since the era of serological screening, asymptomatic cases are now the commonest form of the disease and are 7 times more common than clinically diagnosed patients.

Prevalence of anemia in celiac disease

Because of the improvement in diagnostic methods for identifying celiac disease, there has been a marked increase in the proportion of subjects identified as celiac patients, who do not have the classical manifestations of disease such as diarrhea, gross symptoms of malabsorption including steatorrhea and abdominal complaints. Anemia without other clinical clues of intestinal malabsorption is one of the most common extraintestinal manifestations of celiac disease.[6,7] Although folate and cobalamin deficiency are known complications of celiac disease, the most common nutritional anemia associated with celic disease is iron deficiency. Iron deficiency anemia was reported in up to 46% of patients with subclinical celiac disease in one study, and its prevalence was higher in adults than in children. Similarly, among patients identified by population screening, consisting mostly of young or middle-aged adults, 50% were ane-

mic. In an additional study consisting predominantly of young females, anemia was encountered in 28% of celiac patients and was the most common extraintestinal finding. A characteristic feature of iron deficiency anemia (IDA) associated with celiac disease is its refractoriness to oral iron treatment.

Prevalence of celiac disease in anemia

If anemia is a common presenting feature of celiac disase, what is the chance of encountering celiac disease in patients presenting with iron deficiency anemia? This question is of particular importance for hematologists who are often the first experts consulted for unexplained iron deficiency anemia. Table 1 describes some of the studies focusing on this question. The population of patients studied was heterogeneous as some groups consisted only of patients with iron deficiency whereas others included both folate and iron deficient patients. Most groups consisted of a majority of premenopausal females. In 4 studies[8,11,13,14] small bowel biopsy was only performed in patients with positive serology for celiac disease whereas in 3 other studies[9,10,12] biopsies were performed upfront without being preceded by serological screening. Nevertheless the results were remarkably uniform with *de novo* diagnosis of celiac disease in 5-6% of patients presenting with iron deficiency anemia. Our prospective study of IDA published in 2005[13] has now been extended from 150 to 325 patients. The prevalence of celiac disease remained unchanged at 5.2%. The 17 celiac patients in this series were indistinguishable from the rest of the anemic patients by their age, severity of iron deficiency anemia, or serum albumin. Serum cholesterol 151±36 was significantly lower in celiac disease than in the other IDA patients (180±41, p=0.007). Only one patient had diarrhea, one had stunted growth and 2 had constipation. The most consistent clinical feature was complete refractoriness to oral iron treatment. and the complete absence of an increment in serum iron two hours after oral iron loading with 100 mg ferrous sulphate.

Mechanism of anemia in celiac disease

A number of causative factors deserve consideration for explaining the mechanism of anemia in celiac disease.

Abnormal iron absorption

The most obvious cause of anemia in celiac disease is impaired absorption of iron and other nutrients including folate and cobalamin. Villous atrophy of the intestinal mucosa is an important cause of abnormal iron absorption and this is reflected in the clearcut laboratory evidence of iron deficiency anemia in most anemic patients with celiac diasease. Abnormal iron absorption is also supported by the failure to increase serum iron following oral iron loading, and refractoriness to oral iron treatment.

Increased blood loss

Occult gastrointestinal blood loss has been detected in about half the patients with celiac disease in one study, and this finding appeared to correlate with the severity of villous atrophy.[15] An important limitation of that study was the use of an indirect guaiac test for detecting bleeding. In a subsequent study employing 51 Cr radiolabeled red blood cells, a daily blood loss exceeding 1.5 mL was detected in only one of 18 subjects studied. Others have also found that the rate of positive occult blood tests in celiac disease is low and does not exceed that in the general population. Thus, the evidence supporting increased fecal blood loss in celiac disease is controversial, and although abnormal intestinal bleeding may occur in some celiac patients, it does not appear to play a significant role in the causation of anemia.

Anemia of chronic disease

As discussed above, pro-inflammatory cytokines play an essential role in the inflammatory and cytotoxic mechanisms involved in the pathogenesis of celiac disease. Such cytokines, in particular interferon-γ (IFN-γ), and IL6, are powerful mediators of hypoferremia in inflammation inducing the synthesis of the iron regulatory hormone hepcidin.[16] Increased hepcidin synthesis in turn is responsible for increased ferroportin degradation and the inhibition of iron release from macrophages and enterocytes leading to the well known abnormalities in iron homeostasis associated with the anemia of chronic disease.[17]

In a study focusing on the clinical features of anemia in celiac disease,[18] Harper *et al*. noted that although serum ferritin was indicative of iron deficiency in the majority of anemic subjects, unexpectedly, in 13% of patients it was increased. Because a gluten-free diet resulted in increased serum ferritin in iron-deficient patients, but decreased ferritin levels in those with pre-

Table 1. Prevalence of celiac disease among patients presenting with iron deficiency anemia.

Study	n	Serology	Biopsy	Celiac %	Comments
Corazza *et al*. 1995[8]	200	+	+	5.0	8.5% if only obscure IDA considered
Carroccio *et al*. 1998[9]	85	–	+	5.8	All refractory to oral iron. 55% females, mean age 48 years
Annibale *et al*. 2001[10]	71	–	+	5.6	72% females, mean age 59 years
Howard *et al*. 2002[11]	258	+	+	4.7	83% females aged 32–74. One folate and 11 IDA
Mandal *et al*. 2004[12]	504	–	+	1.8	Folate deficiency included. Of the 9 celiac patients 5 were >65 years old
Hershko *et al*. 2005[13]	150	+	+	5.3	79% females, mean age 39 years all refractory to oral iron
Carter *et al*. 2008[14]	116	+	+	6.0	All pre-menopausal women, mean age 33 years

viously high ferritins, they concluded that nutritional deficiencies alone do not explain anemia in all cases, and that inflammation appears to contribute in some individuals, as evidenced by the presence of anemia of chronic disease.

In a recent study, Bergamaschi *et al*. decided to focus on the role of anemia of chronic disease in the development of anemia among celiac patients.[19] A peculiar feature in the design of this study was the use of refined *precision instruments* to identify anemia of inflammation. At the outset, and in a follow-up period of one year on a gluten-free diet, they have collected data on serum iron, transferrin, serum ferritin, soluble transferrin receptor (sTfRc), endogenous erythropoietin (Epo) and IFN-γ. Among 65 anemic celiac patients, 45 had uncomplicated iron deficiency anemia, and 2 had cobalamin or folate deficiency. In 11 subjects, anemia of chronic disease alone or in combination with iron deficiency has been identified, a prevalence of 17%. To increase the sensitivity and specificity of these blood tests, Bergamaschi *et al.* employed not only primary data but a combination of findings moving in opposite directions in IDA as against anemia of chronic disease (ACD): (a) the sTfRc/log(ferritin) ratio that increases in iron deficiency and decreases in ACD, (b) the ferritin/transferrin ratio that decreases in iron deficiency and increases in ACD, and (c) the log(Epo) O/P ratio that describes the increase in endogenous serum EPO in proportion to the severity of anemia, a response known to be normal in iron deficiency anemia but blunted in the anemia of chronic disease. The results of these findings are described in Table 2. Compared with a group of 30 non-anemic celiac subjects, 45 of the celiac patients had findings typical of iron deficiency anemia. However, in 11 the findings indicated anemia of chronic disease with decreased sTfRc/log(ferritin), increased ferritin/transferrin ratio, and a decreased log(Epo) O/P ratio implying a blunted EPO response. Remarkably, serum IFN-γ levels in ACD were 12-fold higher than controls, but even in the iron deficient group they were increased 3-fold, indicating that some degree of inflammation might have been present in all anemic celiac patients. Hepcidin is an important indicator of ACD. By contrast, correlation of iron status with prohepcidin is limited. The use of a prohepcidin assay instead of direct hepcidin measurements may explain the failure to demonstrate significant differences in prohepcidin levels among the three groups in the above study. An encouraging aspect of the Bergamaschi study was that after one year on a gluten-free diet, the response was equally favorable in the IDA and ACD subjects indicating that the suppression of inflammatory intestinal changes by a gluten-free diet improves anemia both by correcting iron and vitamin malabsorption as well as by abolishing the mechanisms responsible for the anemia of inflammation.

Table 2. Distinction between anemia of chronic disease, iron deficiency anemia and non-anemic patients with celiac disease.

Variables	*ACD (n=11)*	*IDA (n=45)*	*Non-anemic (n=30)*
sTfRc/log(ferritin)	0.56±0.34	2.93±1.99	1.20±0.65
ferritin/transferrin	1.874±4.80	0.027±0.028	0.347±0.596
log(Epo) O/P ratio	0.60±0.27	1.01±0.23	n.d.
IFN-γ (pg/mL)	41.9±27.3	10.72±23..2	3.3±3.9

ACD: anemia of chronic disease; IDA: iron deficiency anemia. Values are reported as mean ± 1 SD (adapted from Bergamaschi et al.,[19] permission pending).

Anemia of celiac disease: a paradigm of anemia in gastrointestinal disease

The study of Bergamaschi *et al.* sets new standards for the clinical evaluation of anemia associated with gastrointestinal disease. By comparison with celiac disease, the role of ACD rather than iron loss may be more critical in other inflammatory conditions of the gastrointestinal tract, in particular inflammatory bowel disease. As inflammatory bowel disease often escapes initial detection by routine endoscopy and colonoscopy, recognizing the features of ACD when the presenting finding is unexplained anemia is a useful clue to the identification of an underlying inflammatory condition. Autoimmune gastritis and *H. pylori* gastritis are also increasingly being recognized as important causes of unexplained refractory iron deficiency anemia in children and adults.[10,13] The diagnosis of autoimmune gastritis and *Helicobacter* gastritis is greatly facilitated by the advent of inexpensive and simple serological screening tests. Autoimmune gastritis is the cause of iron deficiency and/or of cobalamin deficiency in 30% of patients presenting with unexplained refractory iron deficiency anemia. Thus, the likelihood of encountering autoimmune gastritis in patients presenting with obscure iron deficiency is 5 to 6 times higher than of celiac disease. Similar to celiac disease, acute or chronic inflammation of the gastric mucosa is a prominent feature of gastric histology in these conditions. It is quite possible that, in addition to iron deficiency caused by abnormal absorption due to impaired gastric acidity, ACD may play an important role in the pathogenesis of anemia associated with autoimmune gastritis and *H. pylori* gastritis.[20]

For the clinical hematologist, an important take home message is that in obscure iron deficiency anemia, absence of the crude markers of ACD, such as increased CRP, sedimentation rate or fibrinogen may not allow the exclusion of an underlying inflammatory gastrointestinal disease, such as celiac disease and probably chronic autoimmune and/or *H. pylori* gastritis. The sensitive and accurate indicators employed by Bergamaschi *et al.* may facilitate diagnosis and the identification of an underlying inflammatory condition that may explain anemia and guide its effective treatment.

References

1. Pavelev WF. From Aretaeus to Crosby: a history of coeliac disease. Br Med J 1988;297:1646-9.
2. van Heel DA, West J. Recent advances in coeliac disease. Gut 2006;55:1037-46.
3. Hüe S, Mention JJ, Monteiro RC, Zhang S, Cellier C, Schmitz J, et al. A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease. Immunity

2004;21:367-77.
4. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ a/b heterodimer. J Exp Med 1989; 169:345-50.
5 Chorzelski TP, Beutner EH, Sulej J, Tchorzewska H, Jablonska S, Kumar V, et al. IgA anti-endomysium antibody. A new immunological marker of dermatitis herpetiformis and coeliac disease. Br J Dermatol 1984;111:395-402.
6. Halfdanarson TR, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. Blood 2007;109:412-21.
7. Bottaro G, Cataldo F, Rotolo N, Spina M, Corazza GR. The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases. Am J Gastroenterol 1999; 94:691-6.
8. Corazza GR, Valentini RA, Andreani ML, D'Anchino M, Leva MT, Ginaldi L, et al. Subclinical coeliac disease is a frequent cause of iron-deficiency anaemia. Scand J Gastroenterol 1995;30:153-6.
9. Carroccio A, Iannitto E, Cavataio F, Montalto G, Tumminello M, Campagna P, et al. Sideropenic anemia and celiac disease: one study, two points of view. Dig Dis Sci 1998; 43:673-8.
10. Annibale B, Capurso G, Chistolini A, D'Ambra G, DiGiulio E, Monarca B, et al. Gastrointestinal causes of refractory iron deficiency anemia in patients without gastrointestinal symptoms. Am J Med 2001;111:439-45.
11. Howard MR, Turnbull AJ, Morley P, Hollier P, Webb R, Clarke A. A prospective study of the prevalence of undiagnosed coeliac disease in laboratory defined iron and folate deficiency. J Clin Pathol 2002;55:754-7.
12. Mandal AK, Mehdi I, Munshi SK, Lo TC. Value of routine duodenal biopsy in diagnosing coeliac disease in patients with iron deficiency anaemia. Postgrad Med J 2004;80:475-7.
13. Hershko C, Hoffbrand AV, Keret D, Souroujon M, Maschler I, Monselise Y, Lahad A. Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia. Haematologica 2005; 90:585-95.
14. Carter D, Maor Y, Bar-Meir S, Avidan B. Prevalence and predictive signs for gastrointestinal lesions in premenopausal women with iron deficiency anemia. Dig Dis Sci 2008 May 9. [Epub ahead of print]
15. Fine KD. The prevalence of occult gastrointestinal bleeding in celiac sprue. N Engl J Med 1996;334:1163-7.
16. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004;113:1271-6.
17. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;352:1011-23.
18. Harper JW, Holleran SF, Ramakrishnan R, Bhagat G, Green PH. Anemia in celiac disease is multifactorial in etiology. Am J Hematol 2007;82:996-1000.
19. Bergamaschi G, Markopoulos K, Albertini R, Di Sabatino A, Biagi F, Ciccocioppo R, et al. Anemia of chronic disease and defective erythropoietin production in patients with celiac disease. Haematologica 2008;93:1785-91.
20. Beutler E. Hepcidin mimetics from microorganisms? A possible explanation for the effect of Helicobacter pylori on iron homeostasis. Blood Cells Mol Dis 2007;38:54-5.

Treatment of chronic myeloid leukemia in blast crisis

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Blast crisis (BC) is the sword of Damocles hanging over every patient with chronic myeloid leukemia (CML). In the past, CML virtually always progressed to BC. Conventional treatment of BC has been notoriously unsatisfactory resulting in only transient response and prolonging survival only marginally once BC has been diagnosed. In chronic phase (CP) CML, BCR-ABL tyrosine kinase inhibition (TKI) is highly efficacious and prolongs survival significantly. Progression to BC is slowed down in most patients and possibly prevented in some. The current challenge is how well (or how poorly) TKI improves prognosis after diagnosis of BC, and how we can make best use of the limited options that are available.

In this issue of the journal, Palandri and the GIMEMA CML group report on 92 patients with CML BC under imatinib treatment over a period of six years.[1] This is the longest currently available observation of imatinib in BC. Median survival is, at 7.5 months after diagnosis of BC, about twice as long as with historical controls. After a median observation time of 66 months, 7 patients (8%) are alive, 3 after allogenous stem cell transplantation (allo-SCT). In comparison, 605 BC patients of the German CML Study group treated with conventional chemotherapy or with interferon α (IFN) show a median survival after diagnosis of BC of four months (Figure 1).[2] Only 21 patients (3.5%) are alive after a median observation time of 6.4 years, 15 after allo-SCT. The progress with survival Palandri *et al.* report is modest, but probably real.

Definition of blast crisis

Comparisons like this largely depend on the definition of BC and thereby on the time of its diagnosis. First attempts at the definition of BC date back about 40 years.[3] The generally used definition which underlies virtually all current clinical CML trials (including the ELN management recommendations)[4] rests on at least 30% of blasts in blood or marrow and the demonstration of extramedullary blastic infiltrates. This definition is not supported by biological evidence and is therefore arbitrary. The more recent WHO definition proposes a blast count of 20% in analogy to the definition of AML. This is, however, also arbitrary without any biological evidence. It has been shown that a change of definition, e.g. to a blast count of 20%, would regroup up to 10% of patients. Patients with 20-29% blasts, currently classified as accelerated phase, had a significantly better prognosis than patients with more than 30% blasts.[5] Most clinicians and trialists, therefore, will likely stick with the definition for which they have the most data, observe ongoing research closely, and wait for additional evidence on the biology of BC for a new evidence based definition.