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Chronic myeloid leukaemia: The dangers of not knowing your BCR-ABL1 transcript

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1. Introduction

Chronic myeloid leukaemia (CML) is characterised by the Philadelphia chromosome, which involves a translocation between chromosomes 9 and 22. The resultant *BCR-ABL1* gene fusion encodes a protein with constitutive tyrosine kinase activity [1,2]. Monitoring *BCR-ABL1* transcript levels whilst undergoing therapy with tyrosine kinase inhibitors (TKIs) is considered standard of care for all patients and is critical for appropriate therapy choices [3,4]. Current guidelines recommend 3 monthly measurements of *BCR-ABL1* transcript levels to evaluate response and change treatment as required. Molecular monitoring has now become the foundation of response monitoring during therapy. More than 95% of patients express *BCR-ABL1* transcripts where *BCR* exon 13 and/or exon 14 are fused to *ABL1* exon 2. These have also been described as p210 *BCR-ABL1* transcripts, which is related to the size of the generated protein. Quantitative PCR (qRT-PCR) methods that measure e13a2 and e14a2 transcripts are standardised to an International Reporting Scale (IS) to ensure consistent interpretation of results. Rare CML patients express atypical *BCR-ABL1* transcripts where different *BCR* exons are involved in the fusion, such as exon 1 (described as e1a2 or p190 *BCR-ABL1*), or exons 6, 8, 19 or others. Treatment response for patients with atypical transcripts cannot be measured using the standard molecular methods that are used for p210 *BCR-ABL1*. It has long been recognised that the identification of the precise transcript type at diagnosis is imperative to ensure correct techniques are used for monitoring whilst on TKI therapy [2]. Otherwise, there is a risk of reporting false negative results during treatment.

The rapid *BCR-ABL1* GeneXpert System (Cepheid) is increasingly used for diagnostic testing due to its ease of use and substantially reduced processing time compared with standard qRT-PCR. It measures the typical e13a2 and e14a2 *BCR-ABL1* transcripts and has comparable results to qRT-PCR techniques. Here we report a case of a CML patient with an atypical *BCR-ABL1* e1a2 transcript and a long history of *BCR-ABL1* monitoring using a p190 quantitative PCR (qRT-PCR) assay, who over a 16-month period, switched pathology providers with only GeneXpert-p210 testing performed. False negative *BCR-ABL1* values were generated. These results suggested the patient was responsive to therapy and contributed to a decision to discontinue therapy due to co-morbidities. Haematologic relapse followed. This case highlights the critical importance of using applicable monitoring techniques based on the correct *BCR-ABL1* transcript type in CML.

2. Case report

Diagnosis was made on 16 October 2012 when the patient was referred to haematology for investigation of anaemia with haemoglobin of 108 g/L and concomitant leucocytosis with associated night sweats and weight loss. Table 1 contains complete blood examination results at presentation.

Bone marrow biopsy was performed which morphologically resembled chronic myelomonocytic leukaemia. However fluorescent in situ hybridisation (FISH), using locus specific probes, detected the *BCR-ABL1* gene fusion and cytogenetic analysis detected the Philadelphia chromosome in all metaphases, thus confirming a diagnosis of CML. PCR analysis for *BCR-ABL1* detected the e1a2 breakpoint.

The e1a2 transcript is rare in CML, with an incidence of approximately 1.8% [6,7]. It is associated with a specific disease phenotype, manifesting with monocytosis [8] as seen in our case, and is more likely to be associated with lymphoid or mixed lineage blast crisis should transformation occur. It is classified as high-risk disease and in case reviews, shown to have poor response to tyrosine kinase inhibition [7,9].

The patient was commenced on imatinib on the 25 Oct 2012 and *BCR-ABL1* using a p190 qRT-PCR technique at 3 months was 15%. e1a2 qRT-PCR methods are not standardised to the IS, however, serial monitoring provides an indication of reduction during therapy, which is an indication of the molecular response to TKI [5]. Side effects however required cessation of imatinib on Dec 13 2012, and dasatinib was commenced 15 Jan 2013. Serial qRT-PCR levels were performed 3 monthly and by approximately 12 months, transcript levels were 0.53%, which indicated a substantial reduction of *BCR-ABL1* and a reasonable response to therapy. No cytogenetic analysis was performed

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Table 1
Complete blood examination results at presentation.

Haemoglobin	108 g/L (115-160)
Red cell count	3.7 × 10*12/L (3.6-5.2)
Haematocrit	0.33 (0.33-0.46)
Mean Cell Volume	88fL (80-98)
Platelets	312 × 10*9/L (150-450)
White Cell Count	17.9 × 10*9/L (4.0-11.0)
Neutrophils	10 × 10*9/L (2.0-7.5)
Lymphocytes	2.3 × 10*9/L (1.1-4.0)
Monocytes	4.8 × 10*9/L (0.2-1.0)
Eosinophils	0.54 × 10*9/L (0.04-0.40)
Basophils	0.18 × 10*9/L (< 0.21)

at 12 months. The patient continued treatment with dasatinib for approximately 4 years with continual low but detectable level of e1a2 *BCR-ABL1*. Given the lack of international standardisation for atypical transcripts in CML, documenting major molecular response was not possible.

In February 2017, the patient changed health care provider with ongoing transcript monitoring with an alternative pathology provider. No formal handover process occurred hence the vital information regarding the atypical transcript was not communicated. This also coincided with the development of a pleural effusion, necessitating cessation of dasatinib in October 2017. The patient remained off any form of therapy for 7 months and the new laboratory performed *BCR-ABL1* measurements for p210 using the GeneXpert System during this time. These results were reported as "Not detected". The patient developed signs of haematological relapse in June 2018 with a rising WCC of 27.4 × 10^9/L. This prompted repeat testing of the *BCR-ABL1* level in one of the same laboratories that had previously monitored the patient for *BCR-ABL1* using the Qiagen Ipsogen m-bcr kit. This is a method specifically used for quantitation of the e1a2 *BCR-ABL1* transcript. This confirmed the already suspected CML relapse due to lack of TKI therapy with a very high e1a2 *BCR-ABL1* transcript level of 44%. Fig. 1 demonstrates the trend of the patients qRT-PCR results after diagnosis until relapse.

3. Discussion

Although e1a2 *BCR-ABL1* transcript monitoring is not standardised to an international reporting scale, accurate tracking of serial results is currently recommended to detect loss of response [10]. A significant rise in transcript level may necessitate investigation for TKI resistant *BCR-ABL1* mutations that arise in the *BCR-ABL1* kinase domain [11], unless the patient is known to have ceased therapy. Detection of resistant mutations prompts treatment change, with selection of the appropriate TKI according to the resistance profile of the mutation. Relapse in the absence of TKI therapy prompts TKI restart [4].

Accurate upfront diagnosis of CML transcript type and subsequent standardised monitoring is essential owing to the treatment decisions which hinge on reported results [3,4,12]. The NCCN guidelines for CML recommend upfront conventional cytogenetic analysis on bone marrow to confirm the presence of the Philadelphia chromosome. Alongside this, quantitation of *BCR-ABL1* is required and in cases of p210 transcripts, results reported using the International Scale [13]. At diagnosis, correct identification of transcript type is crucial for ongoing monitoring to ensure avoidance of false negative results with the use of conventional primers or rapid *BCR-ABL1* assays [2,14]. Such stringent guidelines exist around molecular monitoring in CML as it is critical for early detection of relapse and TKI resistance in the hope of preventing progression to blast crisis.

The European Treatment outcome study, (EUTOS) identified seven rare variant fusions with the e1a2 amongst those [10]. Atypical transcripts, such as e1a2 require identification using multiplex PCR and agarose gel, on which the transcripts will be identified based on the size of the amplified bands or Sanger sequencing [1,15]. Subsequent monitoring requires knowledge of the transcript at diagnosis and use of relevant techniques to ensure accurate results. The advent of the new GeneXpert rapid *BCR-ABL1* testing platform has ushered in an exciting new era of automation in molecular laboratory practice, however, also introduces another source of pre-analytic error for laboratories testing samples of patients with CML.

The GeneXpert© System is a cartridge-based platform which utilise microfluidics and qRT-PCR to allow for automated and rapid testing for the common p210 *BCR-ABL1* transcripts (e13/14a2). In order to facilitate inter-laboratory comparisons of results, Cepheid has inbuilt a conversion factor allowing for reporting on the IS scale [16]. A system such as this allows for local *BCR-ABL1* reporting as it does not require the expertise involved in more laborious qRT-PCR methods. Its rapid turnaround of 2.5 h and walk away set up make it an attractive option for many laboratories [17].

4. Conclusion

This case exemplifies the danger of treating patients with CML without appropriate understanding of the monitoring techniques and the implications for patients with atypical transcripts. The *BCR-ABL1* transcript type and the implications for monitoring are fundamental to

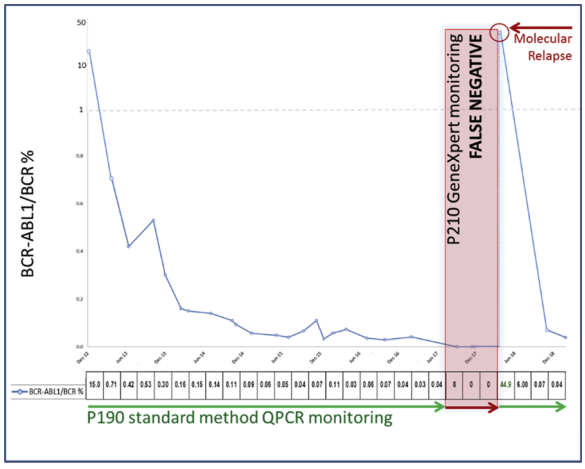

Fig. 1. BCR-ABL1 quantitative values over time from 3 months of imatinib therapy: the percentage e1a2 BCR-ABL1 is shown in the line below the graph. The shaded red region represents the results reported using the GeneXpert system (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

treatment success. We recommend that the transcript type be appropriately characterised at diagnosis and reported with every subsequent *BCR-ABL1* result issued. This information should be conveyed to any new treating clinicians in order that the clinician ensures appropriate monitoring is used for their patient.

Declaration of Competing Interest

Sue Branford is a member of the advisory board of Qiagen, Novartis and Bristol-Myers Squibb and consultant for Cepheid. Received honoraria from Qiagen, Novartis, Bristol-Myers Squibb and Cepheid. Research support from Novartis.

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