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A decade-long analysis of 98 chronic myeloid leukemia patients: Laboratory data and clinical progress at a single center

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Abstract:

BACKGROUND: Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of the Philadelphia chromosome (BCR-ABL1 fusion gene). CML primarily progresses through chronic, accelerated, and blast phases. While global studies on BCR-ABL1 fusion transcript types and their associations with clinical, laboratory, and prognostic profiles exist, such data is scarce in Malaysia.

OBJECTIVES: This study aimed to determine the distribution of BCR-ABL1 fusion transcript types and evaluate their associations with demographic, clinical, laboratory, prognostic profiles, and disease outcomes among Malaysian CML patients.

PATIENTS, MATERIALS AND METHODS: A total of 98 patients diagnosed with CML were identified at East Coast Hospital, Malaysia. This 12-year cross-sectional study was carried out using data extracted from patients' medical records. Molecular results for BCR-ABL1 fusion genes were obtained using one-step multiplex reverse transcriptase polymerase chain reaction.

RESULTS: Out of the 98 patients, 56% had e14a2, 41% had e13a2 fusion transcripts, while the remaining 2 patients had e14a3 transcripts. Additionally, 1 patient co-expressed both e13a2 and e14a2. Among patients with the major BCR-ABL1 transcript type, those with e14a2 fusion transcripts showed an older median age ($P = 0.025$), while patients with e13a2 fusion transcripts had significantly higher white blood cell (WBC) counts ($P = 0.014$). Furthermore, there were significantly more patients with blastic transformation in the e13a2 group ($P = 0.038$). The median latency period of CML was 12 months. The blast cell lineages consisted of myeloid (68.4%), followed by B-lymphoid (26.3%) and mixed phenotypic (5.3%). The differences in fusion transcript expression might influence certain parameters; for instance, older patients were associated with the e14a2 fusion transcript. Meanwhile, patients exhibiting e13a2 might have a higher WBC count at diagnosis and be more vulnerable to blastic transformation of CML.

CONCLUSIONS: This study highlights the predominance of e14a2 fusion transcripts in Malaysian CML patients and its association with older age. The e13a2 transcript was linked to higher tumor burden and a higher rate of blastic transformation, suggesting potential prognostic significance. These findings underscore the importance of baseline molecular profiling for optimizing disease management.

Keywords:

BCR-ABL1, blast transformation, chronic myeloid leukemia, fusion transcript, major breakpoint cluster region

Introduction

Chronic myeloid leukemia (CML) is a Philadelphia chromosome-positive

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myeloproliferative neoplasm, primarily characterized by the proliferation of granulocytes. It accounts for about 15% of adult leukemia with 1–2 incidences per 100,000 people annually.[1,2]

The Malaysian National Cancer Registry Report 2007–2011 recorded a total of 573 cases in Malaysia, representing 12.5% of all leukemia cases in the country.[3] It is marked by a balanced translocation of chromosomes 9 and 22 leading to the formation of the Philadelphia (Ph) chromosome, which harbors the BCR-ABL1 fusion gene. This genetic rearrangement has been a hallmark in diagnosing CML, as more than 90% of CML patients exhibit this recurrent genetic aberrancy.[2] Nevertheless, morphological analysis of peripheral blood film and bone marrow aspirate remains a fundamental investigation to diagnose CML cases.[4]

The Ph chromosome results from the translocation t(9;22) (q34;q11), which fuses the *BCR* and *ABL1* genes to create the *BCR-ABL1* fusion gene. Closer at the molecular level, the breakpoints in the BCR and ABL1 genes are located enclosed by the intronic regions of each gene and are distinctive to each individual. The *ABL1* breakpoints in chromosome 9 are situated in either exon 1 or 2 of the intron. In contrast, the site of *BCR* breakpoints at chromosome 22 can be variable at one of three positions, namely in intron between exons 1–2, 13–15, and 19–20. These regions are referred to as the major-*BCR* (M-*BCR*) being the most common, minor-*BCR* (m-*BCR*), and micro-*BCR* (µ-*BCR*), respectively.[5] Typically, the *BCR* gene breaks down in an intron between exons 13–15 (M-*BCR*). Along with the ABL1 breakpoints, the fusion transcripts are designated e13a2 and e14a2 and both produce a (p210) protein with continuous tyrosine kinase activity [Figure 1].[6]

On the other hand, unusual *BCR-ABL1* transcripts are also found infrequently. These include e1a2 encoding for p190 (involving the m-*BCR*) which is most commonly associated with BCR-ABL1-positive acute lymphoblastic leukemia (ALL) or e19a2 encoding a larger fusion protein p230 (involving the µ-*BCR*).[7-9] Other atypical fusions involving *ABL1* (a3) or other *BCR* exons (e6 and e8) are sparsely described.[4,10]

The disease typically progresses through two to three phases: chronic phase (CP), followed by either an accelerated phase (AP) or a blast phase (BP).[2,5] Most commonly, patients present in the chronic phase (CP), and without effective treatment, the condition can progress to the AP and BP within five years of diagnosis.[11] Instead, with the successful therapy of TKI and rigorous disease monitoring, their overall survival has improved, reaching levels comparable to those of the general population.[2] Thus, in the upcoming 5th edition of the WHO Classification of Haematolymphoid Tumours, AP is no longer required.[12] Clinical presentation can vary, with some patients being asymptomatic at diagnosis while others may present with left upper fullness or pain,

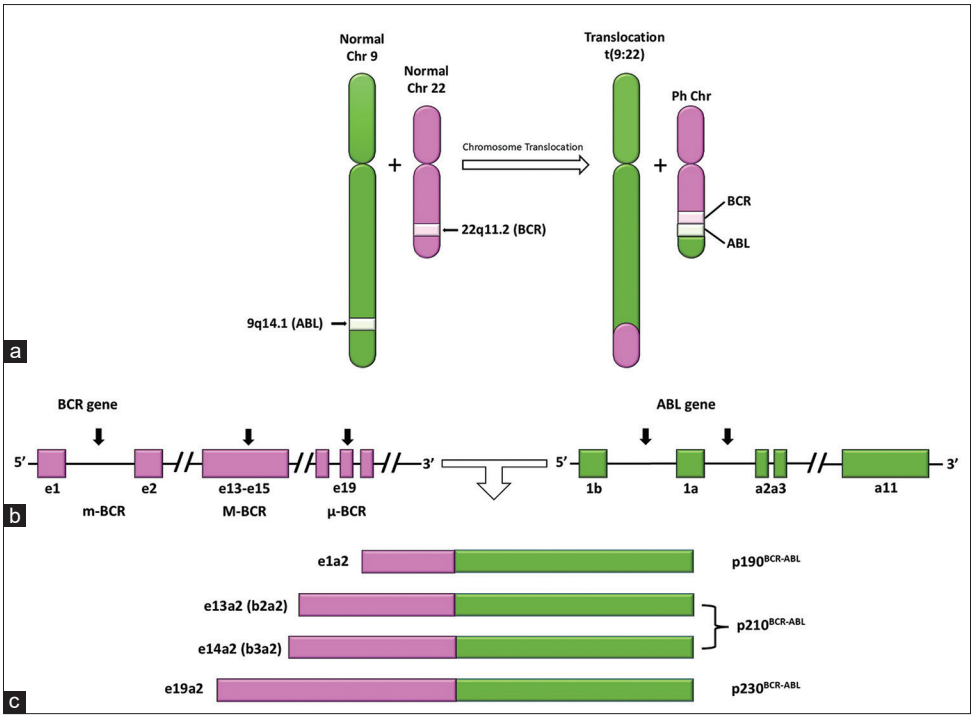

Figure 1: (a) Schematic diagram of Philadelphia chromosome, (b) Breakpoint locations between BCR and ABL1 genes (black arrow), (c) Different fusion protein combinations yield different outcomes

fatigue, weight loss, and fever.[11,13] Approximately 50% of cases present with splenomegaly.[11]

The diagnosis of CML is primarily established through peripheral blood findings combined with the presence of BCR-ABL1.[2] Nonetheless, a bone marrow aspirate is advised for comprehensive karyotype analysis and morphological assessment to confirm the disease phase [Figure 2].[4,10,14] In 95% of CML cases, conventional cytogenetic analysis employing bone marrow cells will identify the Ph chromosome. While, in the remaining cases, reverse transcriptase polymerase chain reaction (RT-PCR) or fluorescence *in situ* hybridization is needed to analyze cryptic BCR-ABL1 fusion.[10] Any additional cytogenetic abnormality (ACA) particularly "major route" abnormalities can signal for increased risk of blastic transformation.[5]

According to European LeukemiaNet (ELN) 2020 recommendations, it is essential to do a qualitative RT-PCR on peripheral blood to detect the specific *BCR-ABL1* transcripts which served as a marker for evaluating the effectiveness of TKI therapy later.[4] Additionally, it is useful for detecting atypical BCR-ABL1 transcripts, while quantitative RT-PCR is not needed for the initial evaluation.[4,7] Around the world, variable frequencies of the fusion transcript in CML patients were reported. Overall, majority of CML cases (97%–98%) exhibited e14a2 and e13a2 fusions. The rest expressed a variety of unusual fusions involving *ABL1* (a3) or other *BCR* exons (often e1, e6, e8, and e19).[10] With regard to the major transcripts e13a2 and e14a2, many literatures suggested mixed opinions on their distribution. Few studies had reported higher e14a2[15-17] than e13a2 while others had shown the opposite.[18,19]

Many literatures also had investigated the influence of these divergent groups on several parameters such as clinical, hematological, prognosis, and treatment outcome. However, their findings were variable.[20] The relationship of e14a2 fusion transcripts with increased platelet (PLT) count was one of the most intriguing findings, with some evidence supporting this association and some evidence contradicting it.[17,21] Meanwhile, other studies discovered patients with e19a2 transcripts had typically pronounced neutrophilic maturation and/or thrombocytosis.[2,22] Previous research had also compared the response of patients with these various fusion transcripts to TKI therapy. Several studies have found that patients with the e14a2 transcript achieve a higher MMR compared to those with e13a2, supporting inferior outcomes in the latter.[23-25] Comparing hematological and cytogenetic response, Kagita *et al*. found in their study that e14a2 may be related to a poor response in CML patients treated with imatinib.[15]

In Malaysia, there is a paucity of data addressing the distribution of various CML fusion transcripts among CML patients. In addition, there is no representative study on associations of types of fusion transcript gene with clinical, laboratory, prognostic profile and outcome of CML patients as yet. Consequently, the main objective of this study is to detect the distribution of fusion transcripts and analyze the associations of major *BCR-ABL1* fusion transcripts with various parameters, including demographic, clinical presentation, laboratory findings, prognosis, and disease outcome in our CML patients.

Subjects and Methods

Study population and study design

This was a cross-sectional study conducted among CML patients diagnosed in tertiary centers, Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II. This study was carried out with ethical approval from the Universiti Sains Malaysia Research Ethics Committee (USM/JEPeM 21030232) and Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR-21-461-58515), in compliance with the Declaration of Helsinki. In this study, 98 patients were recruited from January 2010 to December 2021 to get the distribution of different fusion transcripts among the CML patients. For this purpose, we had included those who were aged 18 years old and above at the time of diagnosis, diagnosed with CML, as described by the

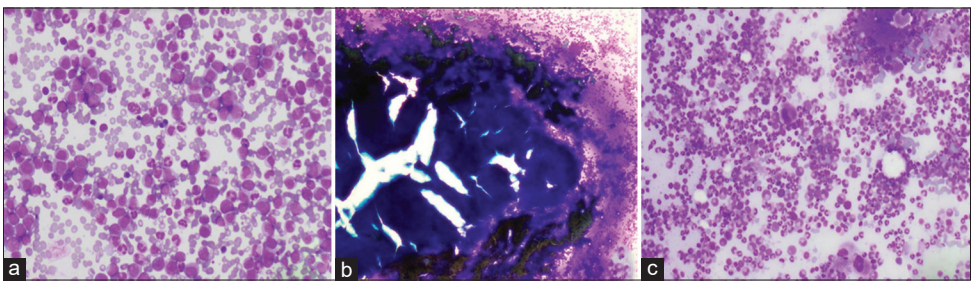

Figure 2: Microscopic and Wright's staining features. (a: HPF, ×20) Peripheral blood of chronic myeloid leukemia patient showed hyperleukocytosis with occasional blast and (b and c: HPF, ×10) Bone marrow aspirate showed hypercellular fragments. There was granulocytic proliferation with peaks in the proportions of myelocytes and segmented neutrophils. No increase of blast cells seen (chronic phase)

criteria of the 2016 WHO classification guidelines and also positive for molecular *BCR-ABL1* with transcript type identified. Meanwhile, the exclusion criteria were CML patients with negative *BCR-ABL1*, pediatric CML patients, and incomplete data.

To correlate fusion transcripts with clinical and laboratory data, prognosis, and outcomes in CML patients, we included only those with major *BCR-ABL1* fusion transcripts, excluding other types such as e14a3 and dual expressions (e14a2 and e13a2). This decision was due to the limited number of patients expressing these less common fusion transcripts. Therefore, out of 98 CML patients identified at the beginning of the study, only 95 patients were studied for the associations. The clinical data obtained were the age, gender, ethnic, clinical presentation, and spleen size. Meanwhile, for the laboratory data, hemoglobin (Hb), white blood cell (WBC), PLT, basophil count, eosinophil count, blast count, bone marrow blast count, cytogenetic study, and molecular *BCR-ABL1* were collected. Finally, for the prognostic profile and disease outcome, Sokal score, transformation, and complete hematological response (CHR) were acquired.

With regard to the molecular results for *BCR-ABL1* fusion gene, the detection of the fusion was carried out by one-step multiplex RT-PCR using RT-PCR kit (titan one tube RT-PCR) in a single tube using automated Thermal Cycler Veriti. Three sets of internal controls were used: a positive control using K562 DNA (e14a2 cell line) and two additional positive controls from known ALL (e1a2) and CML (e13a2) patients. A blank control (NTC) and four sets of external primers, including a control primer *BCR-C* as the internal control, were utilized to simultaneously detect various *BCR/ABL* mRNA PCR products.

The primers used for the amplification of BCR-ABL1 fusion transcripts were as follows:

BCR-C 5′ ACCGCATGTTCCGGGACAAAAG 3′

B2B 5′ ACAGAATTCGCTGACCATCAATAAG 3′

C5e- 5′ ATAGGATCCTTTGCAACCGGGTCTGAA 3′

CA3- 5′ TGTTGACTGGCGTGATGTAGTTGCTTGG 3′.

The amplified products were electrophoresed on a 1.5% agarose gel for 30 min. Finally, the gel picture was visualized under a UV transilluminator (Alpha Innotech, USA) and captured using AlphaImager system (Alpha Innotech, USA). The molecular size used was a 100 bp DNA ladder. The expected band size was 310 bp for e13a2, 385 bp for e14a2, and 481 bp for e1a2. The *BCR* gene was employed as an internal control with a PCR product of 808 bp (*BCR* gene). Figure 3 shows examples of positive PCR products of e13a2, e14a2, and e1a2 while other fusion transcripts are shown in Figure 4.

Statistical analysis

The results were analyzed by (SPSS) (Version 27.0; Armonk, NY, USA, IBM Corp.). Associations were assessed by comparing categorical data using the Pearson Chi-square test or Fisher's exact test. The Mann–Whitney test was used to compare medians between two independent groups. A significance level was set at $P < 0.05$.

Results

Ninety-eight CML patients were recruited for this study. The median age of the patients was 38.5 years, ranging from 18 to 76 years. Among them, 52% were younger than 40 years old. Males made up the majority of them; 69 (70.4%), as opposed to females 29 (29.6%), with an M:F ratio of 2.4:1.0. In terms of ethnic group, almost all of the patients were Malays (91.8%). The distribution of the major *BCR-ABL1* fusion transcripts was as follows: E14a2 was present in 56% of patients, while e13a2 was found in 41%. Only 1% expressed dual transcripts, co-expressing e13a2 and e14a2. Notably, 2% of the patients carried atypical fusion transcripts e14a3 [Table 1].

These associations were studied among CML patients with major *BCR-ABL1* fusion transcripts only $n = 95$. Due to small number of patients, the other three patients who expressed dual transcripts (e13a2 and e14a2) and e14a3 were not included. Table 2 shows that e14a2 type had a significantly higher median of age than e13a2 type, 43 years old compared to 31 years old ($P = 0.025$). No significant association was found with respect to gender, clinical symptoms, spleen size, and phase of disease with fusion transcript types.

Despite the small sample size, we observed a significant difference in the median of WBC count among these two groups ($P = 0.014$). Patients with e13a2 fusion transcripts had a higher median WBC count than e14a2. Although it was not statistically significant, it was noted that the median PLT count in e14a2 was higher than e13a2,

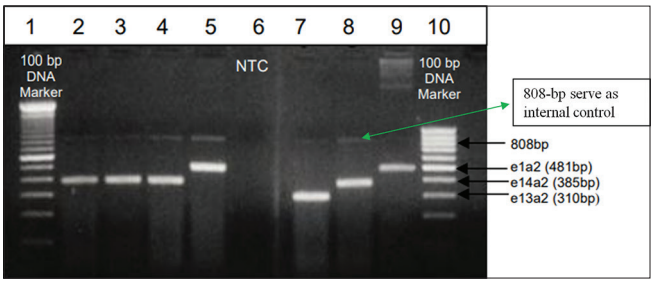

Figure 3: Lane 1 and 10: 100bp DNA maker; Lane 2, 3, 4, 8 e14a2 (b3a2) detected; Lane 5: e1a2 detected; Lane 6: NTC; Lane 7: e13a2 (b2a2) detected; 808bp is internal control band (normal BCR)

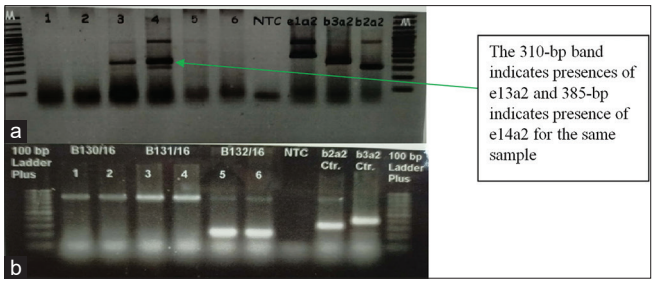

Figure 4: (a) Lane 3 and 4 co-expressions of e13a2 and e14a2. (b) Lane 5 and 6 show *BCR-ABL* gene detected at 210-bp e14a3(b3a3) variant. *Sequencing analysis was proceeded to confirm this variant

Table 1: Descriptive data of chronic myeloid leukemia patients (*n*=98)

Variables	Frequency, *n* (%)
Age (years), median (IQR); range	38.5 (29.0); 17–76
<40	51 (52.0)
≥40	47 (48.0)
Gender	
Male	69 (70.4)
Female	29 (29.6)
Ethnic	
Malay	90 (91.8)
Chinese	7 (7.1)
Siamese	1 (1.0)
BCR-ABL1 fusion transcripts	
Single transcript	97 (99.0)
e13a2	40 (41.0)
e14a2	55 (56.0)
e14a3	2 (2.0)
Dual transcripts	1 (1.0)
e13a2 + e14a2	1 (1.0)

IQR=Interquartile range

529 × 10^9/L versus 410 × 10^9/L. No association was observed between other laboratory data and fusion transcript type. For the cytogenetic analysis, we were able to study a total of 62 patients. This limitation was due to some patients providing suboptimal bone marrow samples and others declining the bone marrow examination.

Table 3 demonstrates the association of fusion transcripts with the prognostic profile and outcome. For this objective, we only studied 95 patients with major *BCR-ABL1* fusion transcripts, while 3 other patients with atypical fusion transcript e14a3 and double expression of e13a2 and e14a2 were excluded. Due to the fact that these fusion transcripts were only found in isolated occurrences, the association might not be representative, therefore their elimination. Patients with e13a2 fusion transcript showed significantly higher cases of blastic transformation, 12 cases (63.2%) compared to 7 cases (36.8%) in e14a2 type ($P = 0.038$). No significant association was observed in regard to Sokal score with fusion type [Table 3]. As for CHR after the initiation of imatinib in 3 months, both groups showed no significant difference. Only 73 individuals in this particular variable (CHR) had their hematological response evaluated; the remaining patients were either sent to another facility after diagnosis or passed away before the 3rd month.

The descriptive characteristics of 19 patients with blastic transformation are delineated in Table 4. Being a tertiary referral center, our population shows a high rate of progression to BP. This group includes patients initially diagnosed with BP, as well as those who began in chronic or APs and later progressed to BP. Majority of the patients were male, 57.9%, and the median age at blastic transformation was 26 years old. The median latency period for disease transformation was 12 months. Among the cases, 68.4% transformed into acute myeloid leukemia (AML), while 26.3% and 5.3% progressed to B-cell acute lymphoblastic leukemia (B-ALL) and mixed phenotype acute leukemia (B/myeloid), respectively.

Discussion

Molecular analyses by RT-PCR have become mandatory during the diagnostic evaluation of CML patients.[26,27] The detection of the *BCR-ABL1* fusion gene and identification of the transcript type at baseline are the current ELN recommendations as part of the diagnostic workup.[4]

In our study, we collected data from 98 CML patients in order to identify the distribution of *BCR-ABL1* transcripts among them. The demographic data of all CML patients indicated a minor gender imbalance, with a higher number of males compared to females, consistent with findings in other studies. The predominance of males could be attributed to the higher prevalence of hematological neoplasms in males compared to females, which may be influenced by genetic, molecular, and hormonal differences.[28] Other factors such as nutrition, smoking, alcohol consumption, and radiation and chemical exposure which were more prevalent in males could potentially be contributors to carcinogenesis.[29,30]

The median age of the CML patients was 38.5 years old which was almost comparable with other studies done in other Asian countries.[29,31] Meanwhile, the median age of CML patients was about 10 years older (45.8–66 years old) in the western population.[16,25,32] Underreporting of geriatric population could be the possible explanation for the younger age group for the low- and middle-income countries. In contrast, in countries with a high standard of living, life expectancy was anticipated to be greater due to the existence of superior medical care services.[33] In addition, the discovery of geographical variations in age at diagnosis shows the existence of a potential environmental component that could influence the

Table 2. Association of major fusion transcripts and demographic, clinical, and laboratory findings in chronic myeloid leukemia patients (*n*=95)

Variables	Fusion transcripts				*P*
	e14a2		e13a2		
	n (%)	Median (IQR)	*n* (%)	Median (IQR)	
Age (years)‡		43.0 (29.0)		31.0 (31.0)	0.025[c]
Gender					
Female	17 (60.7)		11 (39.3)		0.719[b]
Male	38 (56.7)		29 (43.3)		
Presenting symptoms (yes/no)					
Abdominal symptoms	20 (57.1)/35 (58.3)		15 (42.9)/25 (41.7)		0.910[a]
Constitutional symptoms	8 (53.3)/47 (58.8)		7 (46.7)/33 (41.3)		0.697[a]
Fever	6 (42.9)/49 (60.5)		8 (57.1)/32 (39.5)		0.217[a]
Asymptomatic	11 (78.6)/44 (54.3)		3 (21.4)/37 (45.7)		0.090[a]
Lethargy	2 (40.0)/53 (58.9)		3 (60.0)/37 (41.1)		0.647[b]
Headache	3 (75.0)/52 (57.1)		1 (25.0)/39 (42.9)		0.636[b]
Priapism	0/55 (59.1)		2 (100.0)/38 (40.9)		0.175[b]
Spleen size (cm‡)		10.0 (16.0)		11.0 (15.0)	0.859[c]
Phase at initial diagnosis					
Chronic	50 (58.1)		36 (41.9)		0.741[b]
Accelerated	4 (66.7)		2 (33.3)		
Blastic	1 (33.3)		2 (66.7)		
Hb (g/dL)		9.30 (3.30)		9.10 (3.00)	0.603[c]
WBC (×10^9/L)		179.00 (156.40)		296.10 (297.80)	0.014[c]
PLT (×10^9/L)		529.00 (539.00)		409.50 (395.80)	0.164[c]
Basophils (%)		3.80 (4.60)		5.00 (6.10)	0.434[c]
Eosinophils (%)		2.00 (4.00)		2.00 (2.80)	0.624[c]
Peripheral blast count (%)		4.00 (6.00)		3.00 (5.00)	>0.999[c]
Bone marrow blast count (%)		2.00 (3.70)		3.00 (4.20)	0.917[c]
Cytogenetic study*					
Ph chromosome	27 (58.7)		19 (41.3)		0.423[a]
Variant Ph	1 (25.0)		3 (75.0)		
Ph + ACA	7 (58.3)		5 (41.7)		

*n=62, ‡Enlargement below the left costal margin, [a]Pearson Chi-square test was applied, [b]Fisher's exact test was applied, [c]Mann–Whitney test was applied. IQR=Interquartile range, Hb=Hemoglobin, WBC=White blood cell, PLT=Platelet, ACA=Additional cytogenetic abnormality, Ph=Philadelphia

pattern of CML. Potential etiological factors include agricultural and occupational exposures, which have been considered as possible contributors, but no definitive link has been established.[34]

All studied CML patients presented with a *BCR* breakpoint in M-*BCR* region which was translated into p210$^{BCR\text{-}ABL}$ protein. Majority of them expressed e14a2 and e13a2 fusion transcripts involving 56% and 41% of patients, respectively. Consistent with most previous studies,[15,16,21,23,24] our data demonstrated a higher prevalence of the e14a2 transcript among CML patients. Our findings were similar to a study by Deb *et al*. and Sazawal *et al*.[23,35] In contrast, other studies have also noted a greater occurrence of the e13a2 transcript, albeit less frequently. A previous study found that 94.6% of their 40 CML patients carried e13a2 transcript.[19] Meanwhile, almost equal proportions of these fusion transcripts were noted in a study by Jain *et al*. They found that among 481 of their CML patients, 42% had e13a2 and 41% had e14a2.[25]

The variation in frequencies may be attributed to the genetic composition of populations with different ethnicities[18] and the sample size of the study. Sample size should not be disregarded as the larger the sample size might result in more representative and accurate results.[36] Besides, difference in the sensitivities of the techniques used to detect the fusion transcripts should be strongly taken into consideration.[37] Generally, RT-PCR method enabled a single leukemia cell to be detected out of 10^5–10^6 normal cells.[38,39] In our study, the detection of *BCR-ABL1* fusion gene was performed by one-step multiplex RT-PCR. This method has streamlined and abbreviated the RT-PCR process for detecting p210 and p190 in one reaction.[40] Advancements in multiplex nested PCR, combined with high-quality RNA, have significantly improved detection sensitivity, achieving a detection limit of 10^{-9} µg.[41]

Our study revealed e14a2 and e13a2 co-expression in only one (1%) patient out of 98 patients analyzed. Other researchers discovered a range of 0%–16% of such

Table 3: Association of major fusion transcripts and prognostic profile and outcome in chronic myeloid leukemia patients (*n*=95)

Variables	Fusion transcripts		*P*
	e14a2, *n* (%)	e13a2, *n* (%)	
Sokal score			
Low	4 (33.3)	8 (66.7)	0.146[a]
Intermediate	18 (66.7)	9 (33.3)	
High	33 (58.9)	23 (41.1)	
ELTS score			
Low risk	12 (52.2)	11 (47.8)	0.571[a]
Intermediate risk	16 (66.7)	8 (33.3)	
High risk	27 (56.3)	21 (43.8)	
Transformation to blastic phase			
Yes	7 (36.8)	12 (63.2)	0.038[a]
No	48 (63.2)	28 (36.8)	
CHR*			
Yes	34 (56.7)	26 (43.3)	0.747[a]
No	8 (61.5)	5 (38.5)	

*n=73, complete hematological response, [a]Pearson Chi-square test was applied, [b]Mann–Whitney test was applied. CHR=Complete hematological response, ELTS=EUTOS longterm survival (ELTS) score

Table 4: Characteristics of chronic myeloid leukemia patients with transformation to blastic phase (*n*=19)

Variables	*n* (%) or median (IQR)
CML phase at initial diagnosis	
Chronic	13 (68.4)
Accelerated	3 (15.8)
Blastic phase	3 (15.8)
Age at blastic transformation*	26.0 (28.0)
Gender	
Male	11 (57.9)
Female	8 (42.1)
Ratio (male:female)	1.4:1.0
Latency (months)*	12.0 (17.0)
Hematological parameters*	
TWC ($\times 10^9$/L)	66.3 (209.3)
Hb (g/dL)	8.6 (5.2)
PLT ($\times 10^9$/L)	101.0 (91.5)
Blast count (%)	30.0 (51.5)
Bone marrow blast count (%)	23.1 (34.3)
Blast cell lineage	
Myeloid	13 (68.4)
Lymphoid (B-ALL)	5 (26.3)
Mixed phenotype	1 (5.3)
Cytogenetic study (*n*=11)	
Ph only	4 (36.4)
Ph + ACA	7 (63.6)
Molecular study	
e14a2	7 (36.8)
e13a2	12 (63.2)
Isolated extramedullary disease	2 (10.5)
Overall survival (months)*	24 (39.0)

*Median (IQR). CML=Chronic myeloid leukemia, Hb=Hemoglobin, PLT=Platelet, ACA=Additional cytogenetic abnormality, IQR=Interquartile range, ALL=Acute lymphoblastic leukemia, Ph=Philadelphia, TWC=Total white cell count

instances.[21] Co-expression of these fusion transcripts occurred as a result of alternative splicing mechanism rather than of two different clones. Alternative splicing is a process where a single gene produces multiple mRNA variants by altering the splicing junctions.[42] As the disease progressed, only one of the fusion transcripts would prevail.[43] Branford *et al*. suggested that this dual transcription occurs in patients with a linked polymorphism within the BCR gene, leading to the activation of a hidden branchpoint. This results in decreased RNA splicing efficiency and skipping of exon 14 (b3) in both BCR and BCR–ABL.[42]

At the worldwide, approximately 2%–4% of CML patients had atypical *BCR-ABL1*.[4] In our study, we found that two of our patients expressed e14a3 transcripts. Sequencing analysis was performed due to atypical band at RT-PCR and confirmed the presence of e14a3 transcript. The unusual a3 fusion may be overlooked because many commercially available fail to detect breakpoints in the ABL gene of intron 1.[44] Consequently, a false-negative result might be produced. Meanwhile, other studies had found a few other atypical *BCR-ABL1* fusion transcripts such as e1a2, e19a2, e19b2, and e13a3 in their CML patients.[17,24,35] These findings underscore the importance of genomic heterogeneity arising from *BCR-ABL* rearrangements.[37]

Associations of fusion transcript types with demographic, clinical, laboratory, prognostic profile and outcome of chronic myeloid leukemia patients

We then looked into the relationships between the different fusion transcripts with their clinical data, laboratory data, prognostic profile, and disease outcome of the patients, focusing only on the major fusion transcript type, which were e13a2 and e14a2. We observed that the patients with e14a2 fusion transcripts showed a significantly higher median age than patients with e13a2. This finding was similar to a study among Sudanese, which concluded that e14a2 transcripts were found in a higher age group of CML patients.[43] No gender preponderance of the transcript type to either female or male gender. However, studies in Pakistan and Iraq discovered that their male CML patients exhibited a greater prevalence of e14a2 transcripts and females had a higher frequency of e13a2 transcripts.[17,21] However, the findings for the Sudanese population revealed the opposite. Osman *et al*. identified a sex-dependent skew in the allocation of BCR-ABL1 transcript types, with female patients dominating the e14a2 type and male patients dominating the e13a2 type.[18] This aligns with the international review by Baccarani *et al*., which analyzed data from 180 centers and found the e13a2 variant to be more prevalent in men (39.2%) than in women (36.2%; $P < 0.0001$).[45]

With regard to clinical presentation, we found no specific associations of any presenting symptoms with the different types of fusion transcripts. However, from our observations,

most of our CML patients presented with abdominal symptoms which were related to splenomegaly. This finding was comparable to the research done by Kuan *et al*. and Chang *et al*. in Sarawak and Pakistan, respectively.[31,46] Besides, we also found that there were no significant associations between spleen size and phase of disease with the types of transcripts.[16,39] However, a study done by Kagita *et al*. showed different results. They found that a significant number of patients with e14a2 transcript type presented in CP, while patients that co-expressed e13a2 and e14a2 presented in acute phase.[15] Despite this, only five patients with double expression of e14a2 and e13a2 were evaluated, which might have affected its reliability.

There were a few disagreements on the potential association of the type of *BCR-ABL1* fusion transcripts on hematological parameters or prognosis. We observed that e13a2 transcripts are associated with a significantly higher WBC count; comparably, a study in Pakistan by Amin *et al*. found patients with e13a2 transcript exhibited a higher mean WBC count of 173×10^9/L in comparison with count of 121×10^9/L in e14a2 patients.[21] A similar observation was reported by Nachi *et al*., where among 67 newly diagnosed CML patients, those with the e14a2 variant had lower WBC counts.[24] These observations reinforced our findings. Conversely, a study among Iraqis found that the WBC count in patients with e13a2 transcript was lower than e14a2 type.[17] A similar finding was reported in Indian population by Kagita *et al*. Nevertheless, their finding did not give a statistically significant association.[15] The higher WBC count associated with the e13a2 fusion transcript may be attributed to increased tyrosine kinase activity. According to Lucas *et al*., samples from e13a2 patients exhibited higher levels of the CT10 regulator of kinase-like adaptor (CrKL), a surrogate marker for BCR-ABL tyrosine kinase activity, compared to samples from e14a2 patients.[47]

Interestingly, we also found that patients with e13a2 patients significantly showed a higher incidence of blastic transformation in comparison with e14a2 carriers, supporting the possibility of higher tumor burden in e13a2 patients. Polampalli *et al*. reported that among 202 CML patients, the presence of the e13a2 transcript was significantly associated with progression to blast crisis.[48] Jain *et al*. identified 21 cases of disease transformation, with individuals carrying the e13a2 transcript accounting for 71% of those cases.[25] In addition, our results corroborated the findings from prior studies that linked patients with e13a2 with unfavorable outcomes. This included a poorer response to TKI therapy (imatinib) when compared to e14a2 CML patients in regard to molecular and cytogenetic response.[23,47] Jain *et al*. found that patients with the e13a2 transcript had a lower cytogenetic and molecular response when treated with 400 mg of imatinib OD. Therefore, they suggested second-generation TKIs as the initial treatment for patients with e13a2 transcripts, except for the fact that this must be proven by prospective research.[25]

Our study found that there was no significant association of PLT count to either the fusion transcript type. This was also observed by Amin *et al*. in their 70 CML patients.[21] However, a greater PLT count in e14a2 was one of the more commonly reported findings in the studies BCR-ABL1 fusion transcripts worldwide.[49-51] The postulated explanation for the greater PLT counts seen in this group was the potential effect of e14a2 fusion transcript on thrombopoietic function.[52] Perego *et al*. suggested that BCR-ABL1 protein's interaction with megakaryocyte integrins could influence the process of megakaryopoiesis. However, in their investigation, they did not make any conclusion whether the alterations can be induced by e14 exon amino acids.[53]

Regarding other laboratory results, analysis of our data found no significant differences in hemoglobin, eosinophil and basophil count, peripheral blast, bone marrow blast, or cytogenetic study between the two groups of CML patients.[16,24] A study done by de Almeida Filho *et al*. summarized that type *BCR-ABL* transcripts have no influence on hematological parameters which verified our findings.[16] In contrast, Kagita *et al*. discovered that patients with e14a2 transcript had a higher peripheral blast count than e13a2 patients, 6.2% versus 3%.[15]

Similar to other studies, the Sokal score had no significant association with either type of fusion transcripts when prognostic scores were calculated.[15,24] In contrast, Deb *et al*. discovered that e14a2 patients have lower risk scores than e13a2 individuals (Sokal and EUTOS score).[35] In accordance with previous research, we discovered that majority of e13a2 and e14a2 fusion transcript patients achieved CHR within 3 months of initiating TKI treatment.[15,35] This result was in disagreement with the findings by Rashid *et al*. whereby they found that e14a2 patients achieved superior complete hematological compared to e13a2 fusion transcript ($P = 0.05$).[27] Even though we did not cover the impact of different fusion transcripts on the cytogenetic and molecular response to TKI treatment in our study, it is noteworthy that in the 2018 systematic review by Ercaliskan *et al*., they found that e14a2 transcript was associated with preceding, profound, and higher molecular response rates in the majority of studies reviewed.[54] Their findings enhanced the inferior response of e13a2 transcript which might support our findings of increase in blastic transformation in this subgroup. Additionally, our study had analyzed the characteristics of CML patients with blastic transformation. These included cases that were initially identified as BP as well as cases that progressed from chronic or AP into BP as defined by the WHO

2016 guidelines. Similarly to a study by Pérez-Jacobo *et al*., our study found that nearly three-quarters were myeloid BP.[55] Clonal evolution with evidence of additional chromosomal aberrations in addition to the Ph translocation was seen in 7 (63.6%) of our patients with a confirmed diagnosis of blast crisis. Four of them showed high-risk ACA as described by ELN[4] which were + 8, −7, +19, and 11q23 in each of the cases. Approximately 80% of patients with BP were known to exhibit ACA;[2,56] however, our study reported a lower incidence.

Conclusions

This study represents a comprehensive analysis of BCR-ABL1 fusion transcript types among Malaysian Chronic Myeloid Leukemia (CML) patients, offering valuable insights into their distribution, clinical correlations, and prognostic significance. The findings revealed that the e14a2 transcript was more prevalent in this population, particularly among older patients, while the e13a2 transcript was associated with higher white blood cell counts and a greater incidence of blastic transformation, reflecting its potential link to a more aggressive disease course.

The results emphasize the importance of baseline molecular profiling for BCR-ABL1 transcript types as part of the diagnostic and prognostic evaluation of CML patients. This approach can facilitate personalized treatment strategies and better monitoring of disease progression. Moreover, the study highlights the need for further investigations with larger cohorts to explore the impact of these findings on treatment responses and long-term outcomes.

As the first study of its kind in Malaysia, these findings provide a foundation for future research and contribute to the global understanding of CML, particularly in populations with distinct demographic and genetic backgrounds.

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Conflicts of interest
There are no conflicts of interest.

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