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التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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HANAA ALY

THESIS FOR PARTIAL FULFILMENT OF
M.D DEGREE IN CLINICAL HAEMATOLOGY

Title: **STUDY AND SURVEY OF BCR-ABL TRANSCRIPT TYPES IN CHRONIC MYELOID LEUKEMIA (CML) YOUNG EGYPTIAN PATIENTS.**

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INTRODUCTION

Chronic myeloid leukaemia (CML) is a myelo-proliferative disorder with the cytogenetic hallmark of Philadelphia chromosome, characterized by a reciprocal translocation t (9;22) (q34;q11). This translocation results when the Abelson (ABL) gene from chromosome 9 merges with the breakpoint cluster region (BCR) gene on chromosome 22, resulting in a BCR-ABL fusion gene on 22q11 that encodes for uncontrolled tyrosine kinase activity. ***(Nagrani et al.,2011), (Kaaren et al.,2009).***

BCR-ABL gene codes for a protein tyrosine kinase (PTK) that is the cause of leukemic transformation of hematopoietic stem cells ***(Quintas-Cardama and Cortes 2009).***

Breakpoints in chromosome 22 are located in major breakpoint cluster region (M-BCR), leading to origin to two transcripts, e14a2 (B3A2) or e13a2 (B2A2) coding for PTK of slightly different length (P210) ***(Melo 1996).*** Much more rarely, the breakpoint is located outside the M-BCR, either in the minor BCR (mBCR), leading to a fusion gene (e1a2) that codes for a similar PTK (P190) which is more common in Philadelphia-positive (ph+) acute lymphoblastic Leukaemia, or in the micro BCR (µBCR) leading to a fusion gene (e19a2) coding for another PTK (P230) that is the molecular marker of a neutrophilic variant of CML. ***(Quintas-Cardama and Cortes 2009).***

Other, rare transcripts have also been reported. Therefore, more than 90% of CML are B3A2 or B2A2. Alternative splicing or mispricing of the primary transcript

can lead to a contemporary expression of both transcripts (B3A2/B2A2), and sometimes also to a detectable co-expression of the e1a2 transcript (P190) ***(Melo J 1996)***.

Both B3A2 and B2A2 code for a protein (P210) with a tyrosine kinase (TK) function, but with a slightly different length (25 amino acids). It is not known if their activity is identical. It has been reported that they may affect the characteristics of the disease and the risk ***(Pfirrmann, et al. 2017)***.

It has not been found that they affect survival, but it has been reported that they affect the rate, the speed, and the depth of the molecular response to tyrosine kinase inhibitors (TKIs) ***(Jain, et al. 2016)***. Moreover, it has been reported that the two may have a different immunogenicity, an important difference that may affect the probability of achieving a deep or a complete molecular remission ***(Tarafdar, et al. 2017)***.

The incidence of B3A2 and B2A2 has been reported in some studies ***(Hanfstein, et al.2014)***, but not in the majority of the studies of treatment with TKIs. It is not known if the incidence may be affected by ethnic, geographic, gender, and age variables. The incidence and the country, region, gender and age distribution of the rare types are not known. Therefore, the true incidence of BCR-ABL transcript types is unknown. The definition of the incidence of the transcript type is a necessary prerequisite to any other study of the biologic and clinical value of the transcript type. ***(Hanfstein, et al.2014).***

The other important risk factors for CML are high doses of ionizing radiation and occupational exposure to

benzene. This is evident from 20–25 fold increase in the incidence of all the leukaemias among atomic bomb survivors. Limited studies are reported to discuss other risk factors for CML. Alcohol, obesity, and adulthood weight gain are reported to play important roles in CML risk. ***(Nagrani et al., 2011).***

The disease is characterized by three phases, namely chronic phase (CP), accelerated phase (AP) and blast crisis (BC). According to the European Leukaemia Net (ELN), the criteria for BC CML are percentage of blasts plus promyelocytes in peripheral blood or bone marrow $\geq$20%, progressive splenomegaly, thrombocytopenia (<100 X 10^3/µL) unrelated to therapy, and karyotypic evolution ***(Baccarani et al., 2013).***

The results of the treatment of CP Ph+ BCR-ABL1+ CML with TKIs are excellent, survival being very close to the survival of non-leukemic individuals ***(Baccarani et al. 2013)***.

Therefore, there is little room for an improvement of survival. However, there is large room for an improvement of treatment-free remission (TFR), that is the major goal of current treatment ***(Baccarani et al. 2013).***

The transcript type is important because it may affect the probability of achieving the deep molecular response that is required to achieve TFR ***(Baccarani et al. 2013)***

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CML has a worldwide incidence of 1-1.5 cases per 100,000 inhabitants. CML constitutes 15-20% of all leukaemias. The median age at diagnosis is 40-60 years, and although it is rare below 20 years, all age groups can be

affected, CML has a slight male predominance ***(Fletcher et al., 2011)***.

CML incidence rates in western countries vary from 0.6 to 2 cases per 100,000 inhabitants ***(Azzazi and Mattar, 2013).***

Highest rates were reported from Switzerland, USA, Italy, Australia, Germany, and UK. Lower rates were reported for Netherlands, Sweden, China, and India. An estimated 24,090 deaths are expected to occur in 2014 in USA. Death rates for Leukaemia have been declining for the past several decades; from 2006 to 2010, rates decreased by 0.8% per year among males and by 1.3% per year among females. ***(Nagrani et al., 2011).***

Little is known about burden and epidemiological information concerning CML in Egypt. There is recent interest to observe incidence and mortality because of advent of new diagnostic and treatment policies for CML. ***(Azzazi and Mattar, 2013).***

Reliable epidemiological information on chronic myeloproliferative disorders (CMPDs), notably Ph+ BCR-ABL-positive CML is rare. Geographic and/or ethnic variations might contribute to the variability of incidences among registries. Prevalence rate has increased by use of tyrosine kinase inhibitors. ***(Rohrbacher et al., 2009).***

In daily clinical practice, some CML management areas are not in line with the current recommendations. Problematic areas are sub-optimal timing of treatment decisions, under monitoring, and unawareness of new molecular monitoring techniques and of beneficial new

tyrosine kinase inhibitors. Median age differs between cancer registries and clinical trials by 10-20 years. Reports of clinical studies underestimate the true age of the CML population. Elderly CML patients are underrepresented in clinical studies and thus have a reduced access to investigational therapies. ***(Baccarani et al., 2013).***

In the last decade, the importance of ethnicity, socio-economic and gender differences in relation to disease incidence, diagnosis, and prognosis has been realized. Differences in these areas have become a major health policy focus in the world. Many studies were undertaken to examine the demographic and clinical features of CML patients presenting initially at medical centers, particularly those which serve an ethnically diverse population. Female CML patients presented with more significant adverse pre-treatment prognostic factors compared to men, but achieve comparable outcomes. Hispanic patients present with lower risk profile CML and achieve better treatment responses compared to non-Hispanic patients as a whole; these ethnic differences are no longer significant when statistical analysis is limited to patients given Imatinib as first-line therapy. These findings are likely reflective of a unique patient population, and also the importance of ethnicity, socio-economic and gender differences in relation to disease incidence, diagnosis, and prognosis. ***(Birnstein et al., 2009).***

Other studies demonstrated that age-specific rate for CML was highest in age group of 55-74 years, although they are lower compared to western populations. Significant reduction in incidence of CML in recent periods might be because of reduced misclassification of leukaemias. The data of CML has to be observed for

another decade to witness reduction in mortality because of changes in treatment management. ***(Gugliotta et al., 2011).***

The incidence of Ph^+ CML increases with age: the reported median age at diagnosis is more than 60 years in epidemiologic registries and of approximately 50 to 55 years in clinical trials. Following the most widely accepted recommendations that set at 65 years the boundary between young and old persons, a relevant proportion of CML patients at diagnosis are "elderly." ***(Gugliotta et al., 2011).***

However, it should be remembered that no definition of elderly based purely on age in the context of onco-hematology is satisfactory: a different "scoring system" would help the proper allocation of patients to an effective, albeit expensive and potentially toxic treatment.

Older age has been considered a poor prognostic factor in patients with CML. The negative impact of age on response rates and long-term survival was observed regardless of the treatment strategy: Busulfan, Hydroxyurea (HU), Interferon-α (IFN-α), and allogeneic stem cell transplantation (SCT). ***(Gugliotta et al., 2011).***

The 2 more widely used prognostic scores for CML, namely, the Sokal and EURO risk scores, proposed and validated before the advent of Imatinib, identified older age as a variable predicting lower response rates and worse outcome. The reasons that underlie the adverse impact of older age on outcome in CML are poorly understood: it is a common notion that toxicities of SCT and IFN-α increase with age; for other forms of treatment, such as Busulfan

and HU, the explanation is much more difficult and elusive. Moreover, it was thought that different biologic features of CML, co morbidities, worse medical care (which leads to delayed diagnosis and/or inadequate follow-up), and other factors in addition to the therapy given may contribute to the negative impact of age in older CML patients. ***(Gugliotta et al., 2011).***

The first Arab Leukaemia Network (ALN) report demonstrated that age-specific rates for CML in Egypt and Arab nations are lower by at least two decades compared to western populations (highest in age group 30-35 years). ***(Azzazi and Mattar, 2013).***

Geographic and ethnic variations contribute to the variability of incidences among CML registries. ***(Oehler, 2013).***

Reliable data concerning response rates to therapy in Arab nations is lacking. CML management areas are not in line with the current recommendations, due to sub-optimal timing of treatment decisions, under monitoring, and lack of molecular techniques or TKIs. Median age differs between cancer registries and clinical trials by 10-20 years. Reports of clinical studies underestimate the true age of the CML depending on the ease of access to medical services that show great diversity in North Africa and Mediterranean region. ***(Azzazi and Mattar, 2013).***

AIM/OBJECTIVES OF THE STUDY

The primary objective is to assess the incidence of different BCR-ABL transcript types, among young Egyptian CML patients in different centers in Egypt to assess the impact of ethnic and demographic variables on disease outcome, particularly according to gender and age.

And also to assess the relationships of different transcript type with the disease characteristics and response to treatment type.

Secondary objectives are:

a) To assess the incidence of transcript types by gender (male, female) and by age (by decades, from 18 to 50 years)

b) To assess the incidence of transcript types in different CML treating centres.

c) To assess the frequency of transcript types according to the source of the data (Clinical Centres, Diagnostic Laboratories, National or Regional or Multi-institutional Registries, academic studies).

CHAPTER (1): CHRONIC MYELOID LEUKAEMIA

Disease Overview

Chronic myeloid leukaemia (CML) is a clonal myeloproliferative expansion of transformed, primitive hematopoietic progenitor cells. It involves myeloid, monocytic, erythroid, megakaryocytic, B-lymphoid, and occasionally T-lymphoid lineages ***(Kaaran et al., 2009).***

CML was the first human disease in which a specific abnormality of the karyotype —Ph chromosome — could be linked to pathogenetic events of leukemogenesis. It was among the first neoplastic diseases in which therapy with a biologic agent (interferon) was found to suppress the leukemic clone and prolong survival ***(Marktel et al., 2003).***

Although heterogeneous, CML is the best-characterized leukaemia at a molecular level, and studies in recent years have helped to define further the molecular events involved in its initiation and progression and to relate such events to clinical manifestations, the course of the disease, and therapeutic interventions ***(Andersen et al., 2002).***

It is characterized by the presence of the Philadelphia (Ph) chromosome produced by the reciprocal translocation t(9;22)(q34;q11) ***(Kantarjian et al., 1999).***

This translocation leads to the generation of a chimeric gene that results from the fusion of the Abelson murine leukaemia (ABL) gene on chromosome 9 with the Breakpoint

cluster region (BCR) gene on chromosome 22. The new leukaemia-specific fusion gene encodes constitutionally activated protein tyrosine kinases (PTKs) of different molecular weights (p185/190, p210, and p230) ***(Jabbour et al., 2007).***

The oncogenic PTK, located in the cytoplasm, is responsible of the leukemic phenotype, through the constitutive activation of multiple signaling pathways that promotes growth and replication through downstream pathways such as Rat sarcoma (RAS), Rapidly accelerated fibrosarcoma (RAF), Janus kinase (JAK), Myc proto-oncogene, and Signal Transducers & Activators of Transcription (STAT) ***(Jiang* et al., 2010).**

Until a little more than a decade ago, drug therapy for CML was limited to nonspecific agents such as Busulfan, Hydroxyurea, and interferon-alfa (INF-α) **(*Ng et al., 2012).***

INF-α led to regression of the disease and improved survival but was hindered by a multitude of toxicities. Allogeneic stem cell transplantation (Allo SCT) was a curative intervention, but carried with it a high risk of morbidity and mortality ***(Deininger et al., 2009).***

The landscape changed dramatically with the development of small molecule tyrosine kinase inhibitors (TKIs) that were shown to potently interfere with the interaction between the BCR-ABL protein and adenosine triphosphate (ATP), blocking cellular proliferation of the malignant clone This "targeted" approach was found to dramatically alter the natural history of the disease, improving 10-year overall survival (OS) from ~20 to 80-

90% ***(McWeeney* et al., 2011).**

Epidemiology

According to the latest US statistics, CML has worldwide incidence of 1-1.5 cases per 100,000 adults, and accounts for ~15% of newly diagnosed cases of Leukaemia in adults ***(Jemal et al., 2010).***

The incidence of CML increases exponentially with age; the median age at diagnosis is 40-60 years according to Surveillance, Epidemiology and End Results (SEER) and Scandinavian data, much higher than reported in single institution series and although it is rare below 20 years, all age groups can be affected ***(Fletcher et al., 2011).***

There is a slight male predominance of 1·4:1. Ionizing radiation in high doses is the only known risk factor ***(Azzazi and Mattar, 2013).***

Neither chemical exposure nor genetic predisposition is thought to play any role in the development of CML and patients may be counselled that this disease is neither preventable nor heritable **(*Tefferi et al., 2011).***

The Cellular Biology of CML

CML is a myeloproliferative disorder. Myeloid progenitor cells expand in various stages of maturation, are released prematurely into the peripheral blood, and home to extramedullary locations. The disorderly expansion of myeloid progenitor cells appears to result from alterations in their proliferative capacity and a shift in the balance between self-renewal and differentiation toward differentiation, increasing the number of progenitor cells and