SUPPLEMENTARY APPENDIX

The impact of category, cytopathology and cytogenetics on development and progression of clonal and malignant myeloid transformation in inherited bone marrow failure syndromes

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 doi:10.3324/haematol.2014.117457
Manuscript received on October 1, 2014. Manuscript accepted on February 10, 2015.
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Supplementary Methods

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The Canadian Inherited Marrow Failure Registry and Inclusion and Exclusion Criteria

The Canadian Inherited Marrow Failure Registry is a multicenter collaborative study, established in 2001, which enrolls all consecutive patients with IBMFSs in Canada. The registry was approved by the Research Ethics Boards of all participating institutions, and includes all 16 pediatric tertiary care centers in Canada and one adult center. We estimate that these centers care for >95% of the eligible pediatric IBMFS population in Canada. This registry is population-based as >90% of the patients in this study are from centers that enroll >80% of the patients at their institutions.

Patients that fulfill specific diagnostic criteria for an IBMFS are recruited by hematologists at each centre.[1] Briefly, patients are considered to have an IBMFS and are enrolled in the registry if they have chronic bone marrow failure in addition to having either a first-degree relative(s) with an IBMFS or associated physical malformations or are less than 1 year of age at presentation or have positive genetic testing. The diagnosis of the specific IBMFS is then established by the site co-investigator, and reviewed and modified at the central registry office at the Hospital for Sick Children, Toronto, according to previously described diagnostic guidelines based on published data.[1, 2] Patients are considered unclassified-IBMFSs if they do not fit the clinical, laboratory and genetic diagnostic criteria of known IBMFSs.[1] The majority of these patients undergo extensive genetic testing, which was negative. Six of the 13 unclassified cases included herein were also tested by a next generation panel of 72 known IBMFS genes (including *RUNX1* and *GATA2*),

which were negative. Genetic testing is performed at the discretion of the referring physician. Patients registered between January 1, 2001 and August 31, 2011 were included in the current study.

Most patients on the registry have primary IBMFSs, i.e. inherited disorders that have hypo-productive cytopenia as a major component, such as Fanconi anemia (FA).[2] A small proportion of patients on the registry have inherited disorders, which typically do not have bone marrow failure as a component of their syndrome (non-primary IBMFSs), but have either bone marrow failure or CMMT at the time of their enrollment on the registry.

Patient information is collected on standardized forms and includes demographics, diagnosis, symptoms, family history, physical malformations, laboratory and genetic tests, imaging studies, treatment and outcomes. Actual reports stripped of identifiers are often included. Outcomes recorded include severe cytopenia(s), severe aplastic anemia, MDS, CMCAs, leukemia, solid tumors, hematopoietic stem cell transplantation (HSCT) and death. Follow-up information is collected on an annual basis. Patient information from all centers are reviewed at the central registry office for consistent and uniform definitions, eligibility and diagnoses. When necessary, information is clarified with the site research team and curated information is entered into a database at the central registry office.

Exclusion Criteria

Patients with the following groups of disorders are excluded from the registry: 1) acquired aplastic anemia, i.e. cases which do not fit the diagnostic criteria of an IBMFS, 2) de novo MDS or therapy related MDS, i.e. cases which do not have an underlying condition that fits the diagnostic criteria of an IBMFS, and 3) de novo leukemia or therapy related leukemia that is not associated with known IBMFSs or an antecedent bone marrow failure phase.

1. Teo JT, Klaassen R, Fernandez CV, Yanofsky R, Wu J, Champagne J, et al. Clinical and genetic analysis of unclassifiable inherited bone marrow failure syndromes. Pediatrics. 2008 Jul;122(1):e139-48.

2. Tsangaris E, Klaassen R, Fernandez CV, Yanofsky R, Shereck E, Champagne J, et al. Genetic analysis of inherited bone marrow failure syndromes from one prospective, comprehensive and population-based cohort and identification of novel mutations. J Med Genet. 2011 Sep;48(9):618-28.

Supplementary Table

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Supplementary Table 1: Diagnostic criteria for myelodysplastic syndrome

The patient should fulfill both criteria:

I. The patient has evidence of MDS by having either:

a. Two of the following features:

- Cytopenia. Levels of no more than one lineage may be concomitantly increased (e.g. combined anemia and thrombocytosis or combined anemia, thrombocytopenia and leukocytosis)
- Dysplasia that fulfils the following criteria
    1. Prominent dysplasia in each affected lineage (>10% of the cells in the affected lineage)
    2. At least two lineages that manifest prominent dysplasia
- Cytogenetic abnormality
    1. Non-constitutional, acquired clonal marrow cytogenetic abnormality that can be detected in at least 2 cells.
- Blasts
    1. 5-29% in the bone marrow or peripheral blood

Or

b. Unexplained refractory cytopenia with marrow that is persistently cellular or hypercellular, when all other possible causes for such a combination were excluded including peripheral destruction of blood cells or hypersplenism. Supportive diagnostic features include an underlying MDS-predisposition syndrome, high fetal hemoglobin, high red blood cell mean corpuscular volume, patchy distribution of clusters ($\geq$10 cells) of erythroid precursors in the bone marrow with increased numbers of proerythroblasts and increased numbers of mitoses.

II. The patient does not have signs of AML as evident by:

- No classical AML cygotenetics: t(15;17)(PML/RARA), t(8;21)(Runx1;ETO), inv(16)(CBFB1/MYH11),t(9;11)(MLL/AF9)

Doubling time in repeat BM in 2-3 weeks characteristic of MDS and not AML

Supplementary Figures

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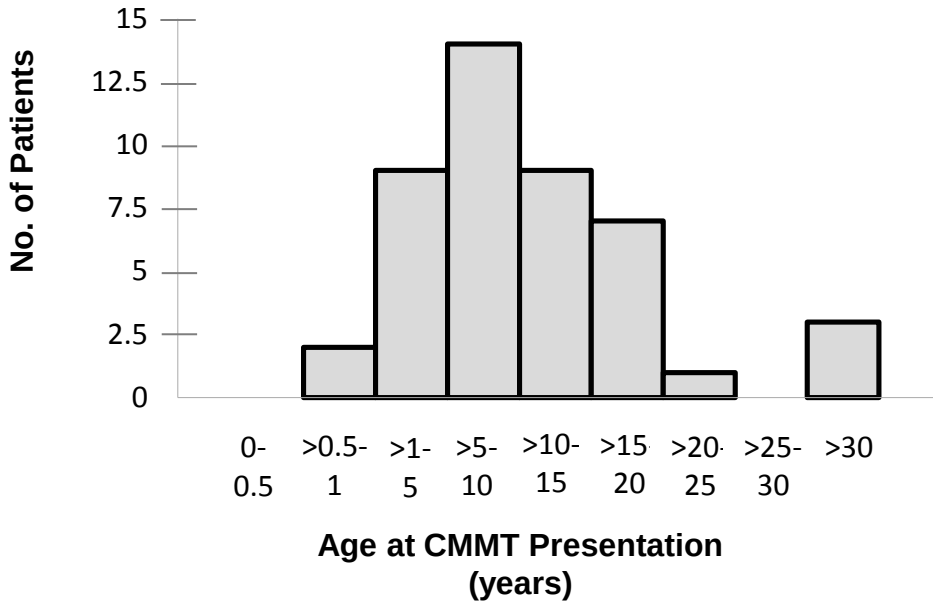
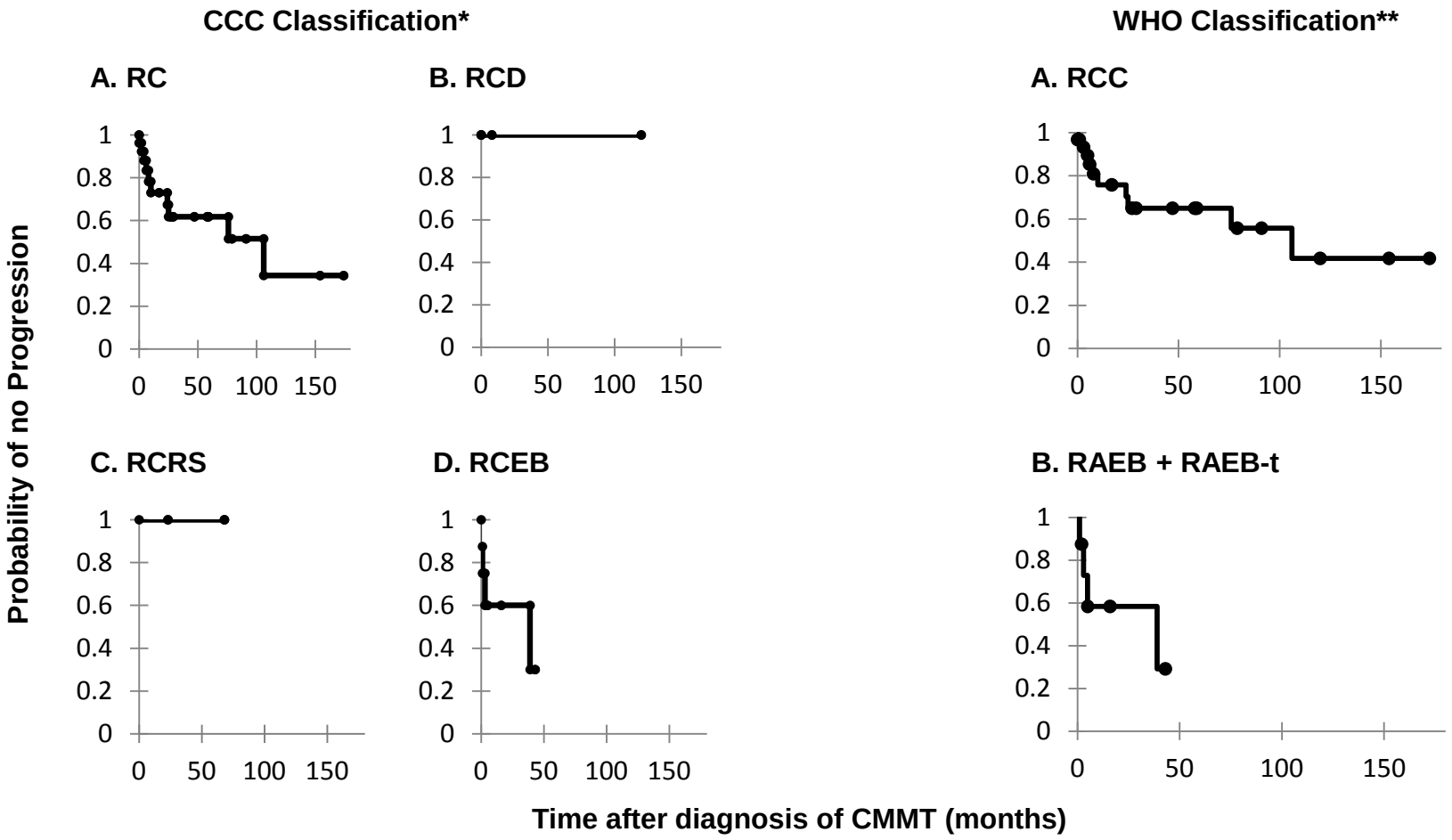
Supplementary Figure 1
Age at Presentation with Clonal and malignant myeloid transformation



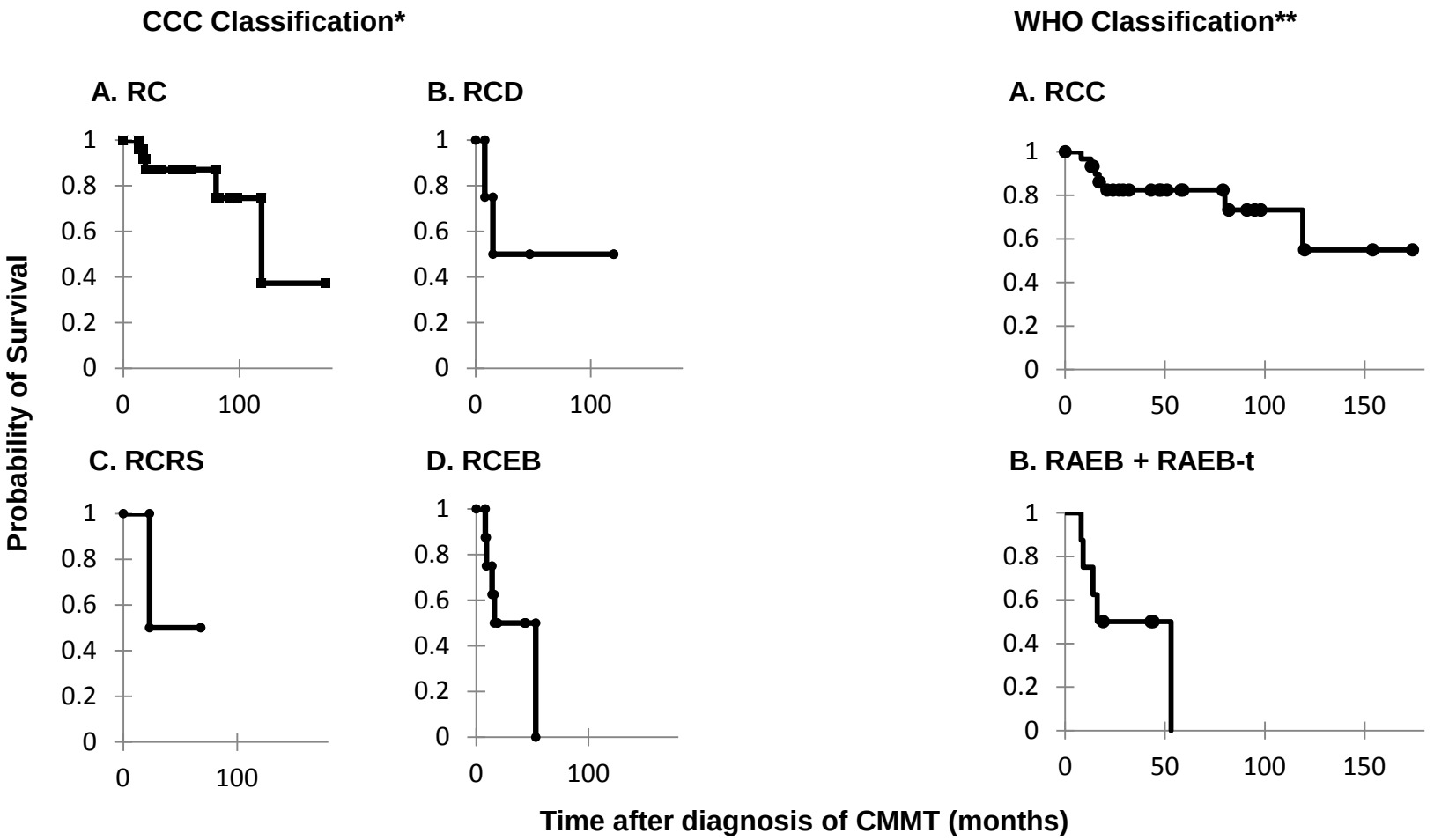
Supplementary Figure 2
Differences in risk of CMMT progression based on differences in the CCC and WHO classifications of childhood MDS



*The Category, Cytopatology, Cytogenetics (CCC) Classification of Childhood MDS: Refractory Cytopenia (RC, myeloblasts < 5%), Refractory Cytopenia with Dysplasia (RCD, myeloblasts < 5%), Refractory Cytopenia with Ringed Sideroblasts (RCRS, myeloblasts < 5%) and Refractory Cytopenia with Excess Blasts (RCEB, myeloblasts of 5-29%).
**The World Health Organization (WHO) classification of pediatric MDS: Refractory Cytopenia of childhood (RCC, myeloblasts < 5%, this includes RC and RCD), Refractory Anemia with Excess Blasts (RAEB, 5-19% myeloblasts) and RAEB in transformation (RAEB-t, 20-29% myeloblasts). We combined RAEB and RAEB-t in our analysis due to relatively small numbers of patients in each category.

Supplementary Figure 3

Differences in overall survival after CMMT diagnosis based on differences in the CCC and WHO classifications of childhood MDS



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