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641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Chromosome Banding and SNP-Array Analysis Are Complementary Methods to Characterize Karyotype Complexity in High-Risk Chronic Lymphocytic Leukemia

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Introduction:
Karyotype complexity is of prognostic significance in chronic lymphocytic leukemia (CLL). It can either be assessed by chromosome banding analysis (CBA) or by chromosome microarray analysis (CMA). How to integrate both methods into CLL diagnostic procedures is not defined.

Material and Methods:
To compare both methods in their ability to detect chromosomal aberrations, samples from 110 patients enrolled on the CLL2O trial (NCT01392079) were analyzed in parallel by Affymetrix ® 6.0 single nucleotide polymorphism arrays (SNP-arrays, CMA) and CBA (CpG oligonucleotide and IL-2 stimulation). The trial included genetically complex high-risk CLL cases defined by *TP53* loss (treatment-naïve or relapsed) or refractoriness to purine-analogue based treatment. Intraindividual reference DNA for paired CMA was available for 79 samples. CMA-defined gains and losses outside known structural variations were counted as aberration irrespective of their size. Discontinuous gains and losses were counted as one aberration whenever segments had a similar log2-ratio.

Results:
CBA identified 561 chromosomal aberrations with a median of 4 aberrations per case (range: 0-21). With 653 DNA copy number alterations, CMA identified more aberrations partly afforded by a high number of unbalanced translocations (median: 5 aberrations per case, range: 0-19). With regards to karyotype complexity (KC), CBA and CMA allocated 67/110 cases (61%) to comparable risk groups as defined by *Baliakas et al. (2019)* and *Leeksma et al. (2021)*. CMA assigned more cases to higher complexity risk groups than CBA, which was not observed when excluding gains and losses <5Mb not previously deemed to be significant. However, this approach did not improve the congruency in KC risk group stratification between both methods (see **Table**).

Only 14/110 cases (13%) had a complete overlap in the chromosomes found to be affected by CBA and CMA. Overall, CBA discovered more additionally affected chromosomes than CMA (p=0.0104). Considering all localizable breakpoints of the CBA-defined structural chromosomal changes (N=922), 532 coincided with a cytoband or a cytoband directly adjacent to one harbouring CMA-defined copy number alterations (N=299 and N=233, respectively), whereas 128 CBA-defined breakpoints located to other cytobands on the same chromosome arm (N=85) or on the other arm (N=43). For 50 of these 128 breakpoints the distance between the CBA-identified cytoband and the CMA-defined breakpoint was <20 Mb.

About one quarter of CBA-defined breakpoints (N=237, 23%) affected chromosomes not found to be altered in CMA. Potential explanations for non-detection by CMA were small clone sizes (78/237, 33%; considering breakpoints found in <25% of metaphases), association with an ultra-complex karyotype (140/237, 59%; referring to cases with ≥10 affected chromosomes and/or a composite karyotype), and no or poor coverage of cytobands by SNP-array probes (72/237, 30%; referring to cytobands with stretches >1Mb covered by ≤5 probes). Besides, the vast majority of the 237 breakpoints belonged to alterations that can in principle be balanced: 209 translocations, 8 insertions and 2 inversions. Only 18/237 breakpoints defined deletions, 15 of which met at least one of the criteria mentioned above as potential explanations for non-detection by CMA.

Overall, 134 chromosomal alterations were detected by CMA alone. Four of these alterations were copy neutral losses of heterozygosity. Most of the 130 DNA copy number alterations were <20 Mb in size (N=104) and 22/130 alterations have previously been associated with a shorter overall survival (affecting 18/110 cases; 16%). Regarding loss of *CDKN2A/ B* and gain of *MYC* as genetic features associated with an increased risk for Richter transformation, CMA identified three cases each lacking abnormalities on the respective chromosome in CBA. CBA identified three cases with a potential *MYC* rearrangement and one case with a potential *CDKN2A/ B* loss despite having normal results for 9p21 in CMA.

Conclusions:

In this cohort of high-risk CLL cases, CBA and CMA were complementary methods for karyotype characterization. CBA was more sensitive in uncovering chromosome involvement than CMA, whereas CMA was superior in determining specific DNA copy number alterations previously associated with an inferior prognosis. As to what extend these findings apply to standard-risk CLL cases needs to be investigated.

Disclosures No relevant conflicts of interest to declare.

Complexity CBA	No. of aberrations	No. of cases identified by CBA	CBA and CMA^{all}	CMA^{all}	No. of CNAs	Complexity CMA
non-CK	*[0-2]*	31	14	19	*[0-2]*	low GC
low-CK	*[3]*	16	11	27	*[3-4]*	intermediate GC
intermediate-CK	*[4]*	11				
high-CK	*[≥5]*	52	42	64	*[≥5]*	high GC

Complexity CBA	No. of aberrations	No. of cases identified by CBA	CBA and CMA^{filtered}	CMA^{filtered}	No. of CNAs	Complexity CMA
non-CK	*[0-2]*	31	19	29	*[0-2]*	low GC
low-CK	*[3]*	16	9	33	*[3-4]*	intermediate GC
intermediate-CK	*[4]*	11				
high-CK	*[≥5]*	52	34	48	*[≥5]*	high GC

Table

Number of cases per karyotype complexity risk group as determined upon CBA and CMA results. For results shown in the lower table, copy number alterations (CNAs) smaller 5 Mb and not deemed to be significant were excluded from the analysis.
(CK = complex karyotype; GC = genomic complexity)

Figure 1

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