Centre of Excellence for Health, Immunity and Infections (CHIP), Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Clonal Hematopoiesis among Older Treated HIV+ Persons Enrolled in the COCOMO Study

AH Borges[1], CW Eskelund[2,3], RF Thudium[4], Y Zhao[4], AD Knudsen[4], M Gelpi[4], C Reilly[5], J Pankow[6] M Polizzotto[7], F Favero[3,8], JH Von Stemann[9], S Ostrowski[9], Klaus Kofoed[10,11], J Weischenfeldt[3,8], K Grønbæk[2,3], SD Nielsen[4] on behalf of the Copenhagen Co-morbidity in HIV Infection (COCOMO) Study Group

[1]CHIP, Dep Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [2]Dep Hematology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [3]Biotech Research and Innovation Centre, Copenhagen, Denmark; [4]Department of Infectious Diseases 8632, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [5]Division of Biostatistics, [6]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA; [7]Kirby Institute for Infection and Immunity, University of New South Wales, Sydney, Australia; [8]The Finsen Laboratory , Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [9]Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [10]Department of Cardiology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; [11]Department of Radiology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

BACKGROUND

Clonal hematopoiesis (CH) is the expansion of blood cell subpopulations containing somatic mutations. CH increases with age and has been associated with death, cancer and cardiovascular disease in the general population.

We set out to

- investigate CH prevalence;
- determine its association with inflammation, T cell subpopulations and coronary calcium among older treated HIV+ persons enrolled in the COCOMO cohort above 55 years.

METHODS

Study population

We used data from the Copenhagen Comorbidity in HIV Infection (COCOMO) Study.

Study Procedures

- Targeted error-corrected sequencing of 21 CH-associated genes was performed in stored buffy coats of COCOMO participants older than 55y.
- IL-1β, IL-2, IL-4, IL-6, IL-10, IL-17A, IFNγ and TNFα levels were measured in plasma using a multiplex assay.
- Flow cytometry identified T cell subpopulations.
- Agatston score was used to quantify coronary artery calcification among participants undergoing a cardiac CT.

Statistical analyses

Cytokine levels, T cell subpopulations and Agatston score were compared between participants with and without CH using standard statistical methods. Multivariable logistic/linear regression identified independent associations.

Mutations	Chr	Hg19-coordinates (only coding regions)
GNB1	1	1,718,773-1,756,892
NRAS	1	115,256,421-115,258,781
ASXL2	2	25,964,901-26,101,091
DNMT3A	2	25,457,151-25,536,853
IDH1	2	209,113,093-209,113,384
SF3B1	2	198,266,558-198,267,764
TET2	4	106,155,095-106,197,673
RAD21	8	117,859,742-117,866,707
JAK2	9	5,073,698-5,073,785
CBL	11	119,077,128-119,170,488
KRAS	12	25,380,168-25,398,318
ETV6	12	11,803,062-12,043,977
IDH2	15	90,631,819-90,631,979
CREBBP	16	3,781,295-3,781,807 and 3,786,120-3,786,204
PPM1D	17	58,740,356-58,740,910
SRSF2	17	74,732,246-74,733,242
TP53	17	7,572,930-7,579,917
ASXL1	20	31,021,087-31,025,138
GNAS	20	57,415,162-57,485,886
BRCC3	X	154,299,803-154,348,422
BCOR	X	39,911,365-39,937,182

Table 1:
CH-associated genes
List of 21 CH-associated genes identified using targeted error-corrected sequencing (Illumina ® TruSeq ® Custom Amplicon)

Characteristics	CH (n=49)	No CH (n=141)	P-value
Age: years, median (IQR)	68 (60-76)	65 (57-73)	0.009
Gender (male) %	98%	83%	0.006
Current smokers (%)	14%	19%	0.662
Alcohol consumption-units, median (IQR)	8(3;14)	7(3;13)	0.745
CD4 at cART initiation, median (IQR) cells/mm³	200 (26;330)	190 (53;261)	0.135
Nadir CD4, median(IQR)	165(110;247.5)	160(72;275)	0.260
CD4:CD8, median(IQR)	0.7(0.5;1.1)	0.7(0.5;1.1)	0.729
Time since HIV infection: years, median (IQR)	22(15;29)	21(17;28)	0.888
cART use: years, median(IQR)	17.5(12.9;19.3)	16.79(12.3;18.8)	0.335
Hepatitis B co-infection (%)	4%	5%	1.000
Hepatitis C co-infection (%)	6%	8%	1.000
Previous AIDS (%)	25%	25%	0.838

Table 2:
Clinical characteristics: COCOMO participants with and without CH
Abbreviations: cART: combination antiretroviral therapy; CH: clonal hematopoiesis; IQR: interquartile range

RESULTS

Clinical characteristics of COCOMO participants with and without CH are presented in **Table 2**

Out of 190 participants (median [IQR] age: 66y [61-70], 87% male, mean CD4+ cell count 678, 99.5% virologically suppressed), 49 (25.8%) had at least one mutation.

In line with previous reports, the most frequent mutations (n/%) were: DNMT3A (25/13.2), TET2 (12/6.3) and ASXL1 (8/4.2) (**Figure 1)**.

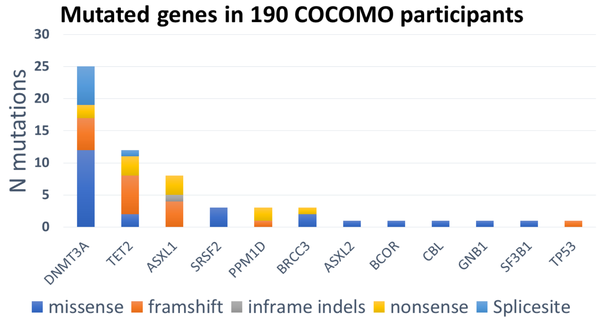

Figure 1: Mutated genes among COCOMO participants. Numbers indicate number of patients with mutations in the specific gene. If same patient has two mutations in one gene, then it only counts for "one mutated gene"

Those with any mutation were older (68 [60-76] vs. 65 [57-73], p=0.009) and more likely to be male (98 vs. 83%, p=0.006). No differences were observed in terms of HIV-related factors (**Table 2**).

Participants with CH had lower IL-10 levels (0.51 [0.29-0.69] vs. 0.58 [0.36-0.89]pg/mL, p=0.049) and tended to have a higher proportion of detectable IL-4 levels (48.5 vs 25.9%, p=0.09); other cytokine levels were similar.

With adjustment for age and sex, CH remained associated with lower IL-10 (adjusted β [95%CI]: -0.10 [-0.20, -0.01], p= 0.03).

Participants with and without CH had similar proportions of T cell subpopulations (p>0.10 for all subpopulations investigated).

Participants with and without CH had similar median Agatston scores (111 [5-357] vs. 76 [0-279], p=0.68). When compared to participants with no mutations, those with TET2 tended to have higher Agatston scores: 232[46-874], p=0.07, but after adjustment for age and sex, TET2 was no longer associated with coronary calcium: β=-0.04 [-0.19, 0.10]; p=0.57 (**Figure 2)**.

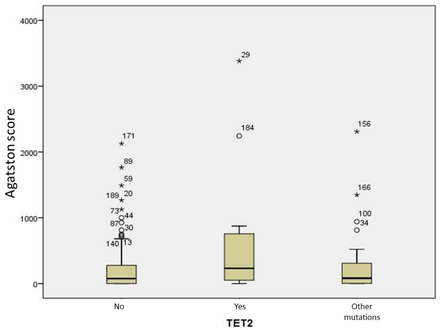

Figure 2: Agatston score in relation to presence of TET2 (n=158)

CONCLUSIONS

- CH is common among older treated HIV+ persons.
- Albeit limited by sample size, our analyses suggest that CH may be associated with dysregulated inflammation.
- Further investigation of CH prevalence and its association with inflammation and coronary calcium among younger treated HIV+ persons is warranted

FUNDING:
Lundbeckfonden (grant R219-2016-762), The Research Council at Rigshospitalet, Augustinus Fonden, Gillead Nordic Fellowship Programme and Danish national research foundation (grant dnrf 126)
COCOMO has been funded by Novo Nordisk, Lundbeck Foundation, The Research Council of University Hospital of Copenhagen Rigshsopitalet, Region Hovedstaden, and Simonsens Fund. In addition, co-funding has been received by the Danish Heart Association, and Åse og Ejnar Danielsen Foundation