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Cardiovascular computed tomography and HIV: The evolving role of imaging biomarkers in enhanced risk prediction

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ABSTRACT

The treatment of human immunodeficiency virus (HIV) with antiretroviral (ARV) medications has revolutionised the care for these patients. The dramatic increase in life expectancy has brought new challenges in treating diseases of aging in this cohort. Cardiovascular disease (CVD) is now a leading cause of morbidity and mortality with risk matched HIV-positive patients having double the risk of MI compared to HIV-negative patients. This enhanced risk is secondary to the interplay the virus (and accessory proteins), ARV medications and traditional risk factors. The culmination of these factors can lead to a hybrid metabolic syndrome characterised by heightened ectopic fat. Cardiovascular computed tomography (CT) is ideal for quantifying epicardial adipose tissue volumes, hepatosteatosis and cardiovascular disease burden. The CVD risk attributed to disease burden and plaque morphology is well established in general populations but is less clear in HIV populations. The purpose of this review article is to appraise the latest data on CVD development in HIV-positive patients and how the use of cardiovascular CT may be used to enhance risk prediction in this population. This may have important implications on individualised treatment decisions and risk reduction strategies which will improve the care of these patients.

Introduction

Since the advent of antiretroviral therapy (ART) people living with HIV (PLWHIV) have had a dramatic increase in life expectancy [1]. This has culminated in patients succumbing to traditional diseases. Cardiovascular disease (CVD) now accounts for the biggest source of morbidity and mortality for this patient group. There is an enhanced risk of CVD in PLWHIV which persists even after adjustment for traditional CVD risk factors compared to HIV-negative populations [2]. This excess risk is thought to be driven by the HIV virus and accessory proteins, chronic inflammation and the increased burden of high-risk behaviours, such as smoking and alcohol excess, which may have a synergistic role in disease development. Some ART regimes have also been implicated in driving excess CVD risk [3, 4].

Cardiac computed tomography (CT) is an imaging technique that has undergone rapid improvement over the last 20 years and as a consequence now has widespread clinical use. Increased temporal resolution from increased gantry spin teams and development of multidetector arrays allow the heart and associated structures to be visualised with very low radiation exposure. The inherent spatial resolution of CT allows for visualisation of coronary arterial walls and atherosclerotic plaque (in addition to luminal stenosis severity). Cardiac CT has become an important research tool in furthering our understanding of plaque morphology and how this relates to CVD risk and acute coronary syndromes.

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Cardiac CT encompasses a wide range of capabilities beyond coronary CT angiography (CCTA). Coronary artery calcium score (CACS) is an established method to quantify an individual's CVD risk. CACS improves traditional risk prediction algorithms and can net reclassify individuals' risk by up to 43% [5]. CACS is approved for use in asymptomatic intermediate risk patients to help guide preventative pharmacotherapy.

Beyond the clinical applications of CCTA and CACS, cardiac CT allows for measurement of epicardial adipose tissue (EAT). This technique can be applied to non-contrast studies, for example when measuring CACS, and can calculate EAT volume. There are multiple vendors that produce post processing semi-automated software that has this capability. Furthermore, the assessment of hepato steatosis (HS) can also be achieved using the non-contrast component of the study.

PLWHIV exhibit a unique hybrid metabolic syndrome that contributes to the increased CVD risk seen. The net effect of this process is efflux of fatty acids away from adipose tissue and increased ectopic fat deposition. This process further drives inflammation and insulin resistance that contributes to vascular injury and atherosclerosis. Cardiac and hepatic tissues are the predominant sites of ectopic fat deposition. The ability to quantify ectopic fat and relate it to an in-depth assessment of atherosclerotic plaque morphology and total plaque burden shows that cardiac CT is an excellent investigation for these patients.

The purpose of this review is to outline the pathogenesis of CVD in PLWHIV and how cardiac CT has been used to gain an insight into the mechanistic processes that underpin this complex interaction.

Antiretroviral Therapy and Development of CVD

Zidovudine was the first antiretroviral (ARV) to market in 1996 and this introduction was associated with a profound improvement in mortality and morbidity. The pre-ARV era (1986–1996) had a 10-year survival of 50% and rate of AIDS-defining illness 30.7/100 patient years [6]. By the end of the 1990s the risk of death reduced by 64% and the rate of AIDS-defining illness reduced to 2.5/100 patient years [7].

Following the dramatic improvement in patient's life expectancy concerns began to arise regarding the significant rates of dyslipidaemia and the potential to contribute to increased CVD risk. HIV infection has been associated with increased total cholesterol (TC) and increased triglyceride (TG) prior to ARV commencement [8]. Treatment with ARV, particularly the early protease inhibitor (PI) formulations, were found to cause significant increases in TC and TG which became more pronounced the longer the duration of ARV. Furthermore, early generation PIs were associated with lipodystrophy and insulin resistance [9]. The Data collection on Adverse events of Anti-HIV Drugs (D:A:D) cohort was developed in response to the adverse effects of ARV and reported their initial findings in 2003. After assessing more 36,000 patient years ARV was associated with a 26% relative increase in the rate of myocardial

infarction per year of exposure [10]. A further report in 2007 demonstrated an increased risk of MI in those taking PI therapy with a doubling of risk approximately every 5 years. The authors conclude the effect was similar to the risk accrued by diabetes or smoking but was not fully explained by the impact on lipids [11]. The risk associated with contemporary PI therapy has been well reviewed elsewhere. Darunavir but not atazanavir seems to be associated with an increased risk of ACS [12].

The D:A:D consortium published a further assessment in 2008 implicating the non-reverse transcriptase inhibitor (NRTI) abacavir with an increased risk of acute myocardial infarction (AMI) [3]. At the time this was unanticipated as abacavir was not known to contribute to dyslipidaemia and therefore a biological plausible link was missing. Further follow up analyses by the same group confirmed these initial findings. Other findings have both agreed and disagreed with these findings. A follow up study by the D:A:D group in 2016 continued to show an association with abacavir and AMI [4].

The incidence of AMI in PLWHIV is between 2 and 7 events per 1,000 patient years [13]. As a consequence, randomised control trials powered to look at hard CVD outcomes are not feasible. The evidence for specific ART regimes is therefore based on large cohort and observational studies. Surrogates for CVD risk have therefore been utilised in order to both evaluate the mechanistic processes involved in HIV and CVD development and determine individual risk.

The mechanistic process of HIV associated CVD

The link between HIV, ART and CVD is in part driven by changes in lipid metabolism, dysfunctional adipose tissue, inflammation and insulin resistance. Metabolic syndrome is an umbrella term that describes a host of deleterious conditions including central obesity, hypertension, insulin resistance and dyslipidaemia. In the presence of a net positive energy state (excess caloric intake and sedentary lifestyle) excess energy is transported to adipose tissue via lipoproteins. Triglyceride is taken up by adipocytes and stored as fatty acids. In the presence of a persistent net positive energy state preadipocytes differentiate to form new adipocytes leading to hyperplastic adiposity. This process is governed by a pre-nuclear enzyme called PPAR- γ . With persistent positive energy balance less preadipocytes differentiate and the remaining adipocytes become hypertrophic as they take up excess triglyceride [14]. This, hypertrophic adiposity, becomes dysfunctional due to ischaemia and lipotoxicity causing secretion of proinflammatory cytokines. The inflammatory cascade interferes with insulin signalling causing the adipose tissue to become insulin resistant. In the state of persistent net positive energy balance there is then an efflux of fatty acids causing ectopic fat deposition. The predominant recipient of ectopic fat is the liver and heart.

Hepatic ectopic fat induces local inflammation and insulin resistance. The liver becomes insensitive to insulin signalling causing increased gluconeogenesis and reduced

glycogen production which can ultimately contribute to development of dysglycaemia. Furthermore, the synthesis of cholesterol is attenuated with a shift towards atherogenic small dense low-density lipoprotein (sd-LDL). This atherogenic lipoprotein carries less antioxidant and is readily glycosylated and oxidised in the presence of inflammation. This phenotype of LDL is also less readily removed from the circulation by the hepatic LDL receptor [15].

The epicardium is a source of ectopic fat deposition and is derived from plastic mesenchymal cells. As the efflux of fatty acids away from adipose tissue continues there is expansion of EAT. EAT is known to exert a paracrine function on myocardium and coronary arteries as they share a common microcirculation. In the state of systemic inflammation EAT becomes proinflammatory and contributes to coronary arterial inflammation via its paracrine function [16]. This positive feedback loop of increasing inflammation contributes to systemic insulin resistance. Inflammation interferes with cellular insulin signalling reducing the effect of insulin on individual cells. There is reduced uptake of glucose via GLUT4 pathways in skeletal muscle and adipose tissue and as a consequence pancreatic β -cells secrete higher amounts of insulin to maintain euglycaemia. In the context of increased insulin secretion cells uptake glucose which can contribute to intracellular glucolipotoxicity. The onset of type II diabetes (T2DM) is when pancreatic β -cells are unable to meet the insulin demands of the tissues [17].

HIV and ARV therapy have unique facets that contribute to coronary atherosclerosis by augmenting this system leading to a 'hybrid metabolic syndrome' Fig. 1. This may be through augmentation of adipocyte function and ectopic fat deposition through a number of mechanisms. The chronic inflammation seen in PLWHIV due to having the virus can contribute to insulin resistance. The HIV accessory protein viral protein R (VpR) suppresses PPAR- γ and reduces the ability of pre-adipocytes to differentiate [18]. In addition, VpR may contribute to development of hepatosteatosis by reducing hepatic fatty acid β -oxidation via interactions with the liver X-receptor [19]. As part of this dysfunction there is loss of association with adipokines and is thought to contribute to lipodystrophy seen in PLWHIV. A further accessory protein, Nef, has been shown to interfere with the normal function of high-density lipoprotein (HDL). It reduces the cholesterol efflux capacity of HDL thus reducing the ability to remove these crystals from arterial walls and contributing to atherogenesis [20]. This effect has also been demonstrated with the PI ritonavir [21].

PI therapy has been shown to interfere with normal insulin signalling by reducing the expression of GLUT4 in skeletal tissues [22]. Older generations of PI have also been shown to have an effect on pancreatic β -cells via voltage gated calcium channels. It blunts the responsiveness to hyperglycaemia reducing insulin secretion. Furthermore, PI therapy can contribute to dyslipidaemia by stimulating the enzyme SREBP1-c which enhances production of apolipoprotein. Older generations of PI have also been shown to inhibit adipocyte differentiation but this effect was not as

pronounced with atazanavir [23]. Certain drugs from the NRTI class have been shown to cause mitochondrial toxicity which further increases chronic inflammation and oxidative stress.

Beyond augmentation of immune dysfunction and inflammation there are adverse effects on the vascular endothelium. The HIV accessory protein Nef has been shown to reduce endogenous nitric oxide production [24]. Ritonavir therapy has also been shown to have a cytotoxic effect [25].

CACS

CACS is a well-established technique for quantifying calcified atherosclerotic plaque within the coronary vasculature. Briefly, this comprises of a gated non-contrast study from the carina to the diaphragm with 2.5 mm slice thickness at 120Kv. The most common quantification method is the Agatston score. This involves quantification of hyperattenuated areas greater than 130 Hounsfield units (HU) within the coronary vasculature which are then weighted according to density and volume. Several well-established cohorts have demonstrated the increasing risk of AMI (and all-cause mortality) with increasing strata of CACS. CACS is a surrogate for overall plaque burden and may be the most important driver for risk (rather than stenosis grading or ischaemia).

CACS was first utilised for assessing CVD in PLWHIV nearly two decades ago. Talwani et al. utilised electron beam CT to assess differences in coronary artery calcium in 60 patients, with 41 being treated with PI [26]. Further work in 2005 demonstrated that HIV patients had a higher amount of CACS above the 90th centile compared to controls [27]. The initial coronary calcium report from the Multicentre AIDS Cohort Study (MACS) suggests that, after adjustment for common clinical covariates, both HIV infection and increasing age had significant odds ratios for the presence of coronary calcium. However, this was tempered by data showing that patients taking ART for greater than 8 years had significantly lower CACS [28]. Sequential studies demonstrated an increased vascular age [29] and increased CACS with HIV-associated metabolic syndrome [30]. However, despite these positive associations a meta-analysis of observational studies by Hulthén et al. concluded that HIV positivity was associated with a small increase in carotid intima media thickening but not the presence of coronary calcification [31].

Interpretation of these early studies looking at the association of CACS and HIV covariates is difficult due to the cross-sectional methodology. Furthermore, the study size was limited in most trials. With the advent of CCTA the focus on CACS as a surrogate for CVD risk shifted to interpretations of plaque morphologies and stenosis quantification.

More recently CACS has been demonstrated to offer incremental accuracy for risk prediction over and above traditional risk scoring methods in HIV-negative populations. CACS of zero is associated with very low risk (<1%) of a CVD event over 10 years. These patients would be unlikely to benefit from statins. Two risk prediction calculators are available that incorporate CACS and traditional

risk factors: the ASTROCHARM calculator and MESA calculator. Use of CACS, particularly in intermediate risk groups, can lead to net reclassification of risk in up to 66% of individuals. This has also led to a recommendation for use in the American Heart Association (AHA) guidelines for asymptomatic intermediate risk patients. The ongoing REPRIEVE study investigating the effect of statin therapy will further help delineate the benefit of statin therapy in PLWHIV [32].

The application of CACS in risk prediction in PLWHIV is limited thus far. Raggi et al. demonstrated that CACS >100 was an independent predictor of CVD events in PLWHIV [33]. Pereira et al. assessed the performance of traditional CVD risk prediction tools and found that the application of CACS reclassified 43.1% of intermediate risk patients [5]. However, CACS is known to underestimate risk in PLWHIV as they have been shown to have higher proportions of non-calcified plaque compared to HIV-negative groups [34].

CCTA

CCTA has undergone significant expansion in both clinical and research realms. Increased gantry spin times, increased detector rows and dual source technology allow the heart to be imaged at end diastole. This reduces motion artefact allowing accurate interpretation of coronary vessels. An iodine contrast injection is administered using either biphasic or triphasic injection protocols. Triphasic protocols includes an initial contrast load, followed by a mix and subsequent saline chaser. This allows sufficient attenuation difference to visualise the right heart. The scan is acquired using ECG gating. Depending on the sequences required the X-ray tube may be active between 50 and 70% of the R-R interval. These developments have enabled high quality coronary imaging at low radiation doses and have been reviewed elsewhere [35].

CCTA has several clinical applications. There are several large multicentred CT registries that have furthered our understanding of the influence of plaque burden on CVD outcomes. The number of obstructed vessels and number of segments with plaque have been shown to be associated with adverse outcomes [36]. CCTA has been shown to demonstrate incremental prognostic information above traditional risk factors and CACS [37].

With the development of CCTA visualisation of non-calcified plaque and vulnerable plaque phenotypes became possible (example of non-calcified plaque in multiplanar reformation in Fig. 2). There are multiple methods to quantify the extent of coronary atherosclerosis. The proportion of those with any plaque, segmental involvement score (SIS), segmental stenosis score (SSS), diameter stenosis (DS) and plaque volumes are commonly having a linear relationship on the risk of future cardiac events. The morphological description of plaque into non-calcified plaque, mixed, calcified and vulnerable plaque (VP) also has implications on the risk of future cardiac events. Non-calcified plaque volume and low attenuation plaque volume have been demonstrated to be associated with increased risk

of future CVD events. VP relates to thin-capped fibroatheroma seen on intravascular ultrasound (IVUS) [38]. The typical appearance on CCTA has been well described previously but include positive remodelling, low attenuation core, spotty calcification and the napkin ring sign [39].

The application of CCTA in PLWHIV has been increasingly used to delineate plaque morphology, plaque burden and CVD risk. The initial studies investigating the relationship of HIV characteristics and CVD centred on CACS – a surrogate for plaque burden. An initial meta-analysis, published in 2015, summarised these early studies showing that HIV infection was associated with >3-fold prevalence of non-calcified plaque but no difference with rates of coronary stenosis or prevalence of calcified plaques. Rates of non-calcified plaque were also associated with lower CD4 cell counts [40]. Further to non-calcified plaque, HIV positive patients have also been demonstrated to have higher prevalence's of vulnerable plaque [41].

Increased rates of non-calcified plaque seen in patients with more advanced disease point towards an inflammatory and immune mediated cause. Increased non-calcified plaque is associated with markers of inflammation even in those with low or undetectable viraemia [42]. A higher burden of non-calcified plaque is also demonstrated in elite controllers, HIV-positive patients who maintain suppressed viral load without ARV's, compared to HIV-negative controls [43]. Given the association of HIV-positivity and non-calcified plaque, unsurprisingly, the MACS cohort have demonstrated an increased prevalence of CACS of zero with non-calcified plaque [34]. This may have an impact on the utility of CACS for risk quantification. Very recently, Pereira et al. demonstrated how use of CACS changed patients risk group [5]. However, given the above studies the extent to which CACS alone can be used to accurately delineate CVD remains to be seen.

Two large cohorts have utilised CCTA in PLWHIV: the Multicenter AIDS Cohort Study (MACS) cohort and the Swiss HIV Cohort. Both cohorts compare HIV-positive patients to HIV-negative patients. Recent publications from both groups suggest different burdens and morphologies of coronary plaque. The Swiss HIV cohort found no significant difference between rates of non-calcified/mixed plaque between HIV-positive and HIV-negative patients with lower calcified plaque in the HIV group [44]. Advanced immunosuppression was associated with non-calcified and mixed plaque and viral load >100,000 was associated with CAC. The MACS cohort found a significant association with all types of plaque type but not significant stenosis [45]. There are obvious and important differences between these two cohorts which may explain the differing results. The Swiss cohort may represent a better treated group with lower rates of smoking and lower rates of HIV-related risks. There was also significant sex (MACS included only men who had sex with men) and racial differences and differences in BMI. A further cohort from Baltimore, USA investigated the presence and extend of coronary plaque in African Americans. They found that coronary plaque was significantly associated with CVD risk calculation and that HIV infection was not

Fig. 1. The physiology of energy transport and how HIV related factors may influence ectopic fat and inflammation.

Panel A: Normal physiology of energy transfer involving lipoproteins. Chylomicrons formed by enterocytes become enriched with dietary triglyceride. They gain apolipoproteins E, CII and B48 which allows them to interact with lipoprotein lipase situated in vascular endothelium (particularly in adipose tissue). As the chylomicron is hydrolyzed and gives up TG to peripheral tissues there is a loss of CII surface markers reducing further interaction with lipoprotein lipase. The resulting chylomicron remnants are cleared by the liver. VLDL is secreted by hepatocytes and are triglyceride rich. Triglyceride is transferred to peripheral tissues via LPL mediated pathways. With continued interaction with LPL there is progressive loss of CII and formation of IDL. Approximately 50% is reabsorbed via LDL-receptor in the liver and 50% undergoes further hydrolysis to form LDL. LDL has a single B100 apolipoprotein and is reabsorbed by hepatic tissues via the LDL-receptor. PPAR- γ is activated in adipocytes which stimulates preadipocytes to differentiate. In the context of a net positive energy balance a hyperplastic adiposity ensues.

Panel B: White adipocyte inflammation. In a constant net positive energy balance adipocytes continue to store excess energy as triacylglycerol. The effects of PPAR- γ are diminished and fewer preadipocytes differentiate and a state of hypertrophic adiposity occurs.

Fig. 2. Example of non-calcified plaque with a calcium score in a patient living with HIV.

Panel A: Multiplanar reformat of LAD showing non-calcified plaque typical of HIV infection.

Panel B: Axial, coronal and sagittal sections of the vessel depicting non-calcified plaque. C: Multiplanar reformat of the dominant RCA showing no significant plaque. The white arrows depict the non-calcified plaque in the LAD.

Panel C: Multiplanar reformat of the RCA

significantly associated with the presence of subclinical CVD. The authors conclude that differences may be due to methodological differences in measurement of non-calcified plaque, older age and worse risk profiles in the MACS cohort compared to the Baltimore cohort [46].

The impact of ARV regimes on atherosclerotic plaque has also been assessed although the data is discrepant. Data from the MACS cohort was published in 2016 showing no consistent significant association between ARV regimes and presence and extent of coronary plaque in well treated patients [47]. Data from the large Swiss HIV Cohort suggested noncalcified and mixed plaque was significantly associated in patients with exposure to abacavir. Negative associations

were found for emtricitabine with noncalcified/mixed plaque, tenofovir disoproxil with any plaque and efavirenz with calcified plaque [48]. Both papers utilised the statistical technique of inverse probability of treatment weighting to adjust for channelling bias for certain ARV regimes. As previously mentioned, important differences between the MACS cohort and Swiss HIV cohort exist including racial, sex, cardiac risks and use of lipid lowering agents. These differences make it difficult to directly compare these two cohorts.

The influence of statin therapy on CVD risk in PLWHIV is of great interest. Traditional CVD risk calculators recommend statin therapy in intermediate and high-risk

As mature adipocytes expand to accommodate the excess triglyceride they become lipotoxic and ischaemic causing inflammation. The inflammatory cascade causes macrophage and T-cell recruitment which further exacerbates the immune response which interferes with adipocyte insulin signalling causing localized insulin resistance. The inflammatory milieu and insulin resistance render the adipocytes unable to take up further excess energy which is subsequently stored as ectopic fat. As cardiac and hepatic ectopic fat accumulates localised insulin resistance and inflammation ensues with resultant end organ damage in the form of coronary atherosclerosis, cardiac contractile dysfunction and cirrhotic liver disease.

Panel C: The Influence of HIV related factors on cyclical nature of maladaptive energy storage. HIV medication attenuated changes include PI induced inhibition of SREBP1-c which inhibits peripheral lipoprotein lipase causing increased free fatty acids. Furthermore, PI therapy may cause increased intranuclear accumulation of SREBP1-c in hepatocytes causing increased VLDL production. 1st and 2nd generation PI therapy have been implicated in reduced expression of skeletal GLUT4 and pancreatic voltage gated calcium channel pathways. This causes reduced capacity of skeletal muscle glucose uptake and reduced pancreatic insulin secretion. NRTI cause adipose cell mitochondrial toxicity causing enhanced oxidative stress and susceptibility. HIV related factors include accessory protein (Tat) induced mitochondrial inflammation and Vpr induced dyslipidaemia. There is also reduced expression of PPAR- γ subcutaneous adipose tissue reducing differentiation of pre-adipocytes. Chronic inflammation induced by HIV contributes to systemic insulin resistance by interfering with insulin signalling pathways. The pressures exerted by both viral processes and ART contribute to maladaptive energy processing and subsequent ectopic fat deposition, insulin resistance and ectopic fat deposition

patients based on LDL-c. Whilst LDL-c lowering is the principle aim of lipid lowering therapy statins are known to attenuate CVD risk via their immunomodulatory and anti-inflammatory effects as well. In 2019 Whelton et al. investigated the relationship between lipids and coronary plaque in the MACS cohort. They concluded that the relationship between atherogenic lipid markers was weaker for HIV-positive patients compared to negative patients. TC/HDL has the strongest association for both HIV-positive and negative patients [49]. The triglyceride to HDL ratio was not reported on despite triglyceride levels demonstrating a stronger relationship across plaque phenotypes and different multivariate models. TG/HDL ratio is also strongly associated with metabolic syndrome and CVD in non-HIV populations. Given that the relationship between lipid indices and coronary plaque seems to differ in PLWHIV it suggests differences in the pathogenesis of coronary plaque. Therefore, traditional risk calculators (which rely on these indices) and specific LDL-c lowering targets may not be as effective in risk prediction in PLWHIV.

The impact of statin therapy and the degree to which it attenuates risk is the subject of the REPRIVE trial which is currently recruiting [32]. Work by Lo et al. in 2015 reported on a randomised control trial investigating the effect of atorvastatin on aortic inflammation and coronary plaque in PLWHIV using serial CCTA. No significant changes were demonstrated in aortic inflammation but there were significant reductions in non-calcified plaque volume and high risk plaque features compared to placebo [50]. Further work by the same group demonstrated that statin therapy has also been demonstrated to reduce HS [51]. A further report by Foldyna et al. assessed changes in coronary plaque on a lesion by lesion basis. They found that statin therapy reduced plaque progression by reducing the fatty and fibrotic plaque components [52]. Similar findings have been found in non-HIV populations, principally from the PARADIGM registry trial [53].

The impact of sex and race on the development of CVD in PLWHIV is under investigated. The MACS cohort is exclusively male while the Swiss HIV Cohort is 85% male. Foldyna et al. compared 48 HIV-infected women to 97 HIV-infected men demonstrating lower prevalence's of plaque and lower VP in women [54]. This may suggest there a sex-specific reduction in cardiovascular risk in PLWHIV although the mechanistic process for this is unknown. In a general population black people are known to exhibit lower calcified plaque compared to other races [55]. There was no significant difference between sexes in the Swiss HIV cohort [44]. In a sub study of the MACS cohort Miller et al. demonstrated lower incidence of calcified plaque and stenosis >50% in HIV-positive black men compared to non-black men [85]. This suggests a similar pattern of atherosclerosis development seen in non-HIV populations.

Hepatosteatosi

Quantification of HS via CT correlates well with biopsy results (the gold standard). Using non-contrast acquisition,

the mean attenuation of the hepatic parenchyma is quantified by drawing two region of interest (ROI) circles (>10 mm²) in the opposing lobes of the liver. A further ROI is drawn on the spleen and a liver spleen ratio of <1 indicates HS (example in Fig. 3). A mean attenuation of <40HU on the liver parenchyma also indicates moderate to severe HS (>30% steatosis) [56, 57]. Quantification of hepatic fat content using the liver/spleen ratio is also possible using contrast enhanced studies, but is less reliable due to differences in scan timing and contrast administration [56, 58].

The prevalence of non-alcoholic fatty liver disease (NAFLD) has dramatically increased in recent years. The current global prevalence is estimated at 25% [59]. NAFLD is closely associated with a host of deleterious metabolic pathologies including hypertension, type II diabetes (DMII) and insulin resistance which is commonly characterised as the metabolic syndrome. The most sensitive clinical predictor of NAFLD is DMII with a high global prevalence of

Fig. 3. Example of quantification of hepatosteatosi in a patient living with HIV from coronary calcium score.

Panel A: Topogram performed prior to non-contrast coronary artery calcium score. The orange line indicates the superior most slice of the study. The green line indicates the inferior most slice of the study and axial slice in which the quantification of hepatosteatosi takes place in panel B.

Panel B: two 100 mm² ROI's drawn in distant (anterior and posterior) regions of the liver avoiding vessels and lesions. A further 100 mm² ROI is drawn in the spleen. The mean hepatic attenuation is 60 HU and mean splenic attenuation is 54. The liver/spleen ratio is >1 therefore there is no evidence of hepatosteatosi

NAFLD in patients with DMII of 55% [60]. The overall prevalence of NAFLD in the United States is predicted to increase to 33.5% of the adult population by 2030 [61].

The histopathological consequence of NAFLD, which encompasses a range of progressive inflammatory hepatic syndromes, is underpinned by HS. Progressive hepatic ectopic fat deposition results in inflammation giving rise to the unique clinical entity of non-alcoholic steatohepatitis (NASH). Continued inflammation induces fibrosis causing cirrhotic liver disease which is strongly associated with liver related outcomes and a threefold increase in mortality [62]. Hepatocellular carcinoma occurs most frequently in those with established cirrhosis [63] but is also increased in NAFLD without cirrhosis compared with the general population [64].

In PLWHIV the prevalence of HS is between 13 and 65% [65–67] and is the most common cause of liver disease [66]. The traditional metabolic risk factors for development of HS in non-HIV populations are common in PLWHIV. Dyslipidaemia and diabetes are a common association with HS [68]. Whilst the relationship of obesity and HS is well defined in non-HIV people it remains more complex in PLWHIV. Lower body mass index (BMI) has been shown to be associated with visceral adiposity and reductions in peripheral fat [69, 70].

HS has been linked with increased subclinical cardiovascular disease [71], increased risk of CVD events [72] and CACS progression [73]. A comprehensive systematic review has demonstrated a positive association with HS and multiple surrogates for CVD including carotid intima media thickness, CACS, endothelial dysfunction and arterial stiffness [74]. HS has also been demonstrated to be a risk factor for CVD on invasive coronary angiography [75]. In contrast several studies have shown no significant association with

NAFLD and CACS [76–78]. The degree to which HS influences CVD risk in PLWHIV is yet to be fully described.

Given that HS shares common CVD risk factors the degree to which it can be considered independently associated remains to be fully elucidated. Many studies investigating this link to not publish the collinearity statistics of their multivariate models. It may be intuitive to think that HS represents an advanced metabolic phenotype or is the hepatic manifestation of metabolic syndrome. The causal role of HS in CVD development is yet to be established beyond the description of known associations of clinical risk factors.

Epicardial Adipose Tissue

EAT lies between the myocardium and visceral pericardium. Embryologically it is distinct from pericardial adipose tissue and they have different vascular supplies. EAT lies directly over the myocardium and adventitia of the coronary vessels. They share a common microcirculation and EAT supplies the adventitia of the vasculature via the vasa vasorum. Physiologically EAT acts as an energy source for the underlying myocardium. It is able to rapidly synthesise fatty acids which acts as the energy substrate of approximately 70% of myocardial energy expenditure. Furthermore, it rapidly absorbs free fatty acids and thus acts in a cardioprotective role from the effects of lipotoxicity.

Beyond its regulation of myocardial energy EAT has several other cardioprotective functions. It provides structural support to the myocardium and coronary arteries. EAT also displays brown adipose tissue functions and protect the heart from hypothermia via non-shivering thermogenesis. EAT also has exerts significant local metabolic regulation by production anti and proinflammatory cytokines and adipokines. The close relationship via a common vasculature

Fig. 4. Quantification of epicardial adipose tissue in PLWHIV.

Panel A: the machine learning derived contours with quantification of epicardial fat volume, and mean density.

Panel B: quantification of regional epicardial fat volumes and densities

allows EAT to exert a paracrine and vasocrine effect on myocardium and coronary vessels.

Quantification of EAT is readily achievable using CT (example in Fig. 4). Software from multiple vendors allows semi-quantitative assessment of EAT volume, distribution and density. Machine learning systems have also been demonstrated to be effective in quantifying EAT. A ROI is drawn around the epicardium. This typically begins at the bifurcation of the pulmonary artery and continues to the apex. Threshold values for detection of fat vary slightly but typically are set to a lower limit of -190HU and an upper limit of -30HU. Quantification of EAT is also possible with contrast enhanced datasets with adjustment of the upper limit to 0HU. Good correlation is also shown between gated and non-gated studies.

Increased EAT volume is associated with coronary artery disease. The switch to a proinflammatory phenotype seen with increasing ectopic fat deposition causes proinflammatory cytokine release which has a direct effect on coronary vasculature via the intimate microcirculation. This promotes endothelial dysfunction and subsequent vascular injury. EAT volume has been associated with increased CACS, increased CACS progression and adverse CVD events. EAT volume has also been demonstrated to be a risk factor independent of visceral adipose tissue and BMI [84].

The role of EAT in PLWHIV is less well defined. Guaraldi et al. demonstrated in a large cross sectional study the association between EAT and CACS >100 [79]. Increasing EAT volume (along with increasing age, male sex and type II diabetes) was independently associated with presence of CACS >100. EAT also had a greater predictive value for the presence of CACS >100 than waist circumference, BMI and visceral adipose tissue although this difference didn't reach statistical significance. In 2014 Brener et al. published findings from the MACS cohort comparing both HIV positive men and HIV-negative men. HIV infection was associated with significantly higher EAT volume after adjustment for CVD risk factors. Further significant HIV associated variable were duration of ARV use, and treatment with atazanavir. Assessment of plaque morphologies demonstrated that both non-calcified plaque and presence of any plaque were significantly associated with EAT after adjustment for risk factors [80].

The effect of EAT in PLWHIV may differ between sex. Srinivasa et al. assessed 55 HIV-positive and 27 HIV-negative women for the impact on EAT on CVD [81]. Whilst both groups exhibited a similar EAT volume in the HIV-negative group the EAT volume was significantly associated with CVD risk scores ($p=0.02$). This association was not present in the HIV-positive group ($p=0.99$). Furthermore, the HIV-positive women had higher non-calcified plaque with the highest percentage in patients with excess EAT. Markers of calcified plaque were highest in the HIV-negative women.

The morphological differences of coronary plaque associated with EAT in PLWHIV may be secondary to the unique effect of the package of HIV risk factors. The 'hybrid metabolic syndrome' may predispose EAT to produce

proinflammatory cytokines and vasocrines. This inflammatory process could give rise to the non-calcified plaque phenotype common in PLWHIV and ultimately contribute to the excess risk. Further work should be forthcoming in defining the role of EAT in HIV populations. It could serve as a risk factor for development of clinically apparent CVD and even a therapeutic target for risk modification.

Intramyocardial fat

Increased intramyocardial fat has been implicated in development of diastolic dysfunction and adverse outcomes in PLWHIV [82]. Increased cardiac steatosis occurs due to the metabolic dysregulation outlined in this review article. Cardiac steatosis is defined as intramyocardial triglyceride content >0.5% and can be measured using magnetic resonance spectroscopy. In a sub study of the Reprieve trial Neilan et al. demonstrated the high prevalence of cardiac steatosis (74%). The significant associations with increased triglyceride were age, raised BMI and prior intravascular drug use [83]. Quantification of intramyocardial fat may be an increasingly important biomarker as a prelude to clinically overt heart failure. It may give enhanced opportunity for lifestyle modification and may represent possible future therapeutic targets to improve morbidity and mortality.

Conclusion

Cardiac CT has proven a useful tool for CVD diagnosis, risk prediction and research purposes in PLWHIV. The burden of HIV associated CVD will increase exponentially over the coming decades as this population succumb to traditional age-related disease. The mechanistic processes involved in the excess risk is still not completely understood. Cardiac CT has been at the forefront of efforts to decipher the roles of HIV-related risk factors. The utility of cardiac CT ensures that this modality will have a clinically relevant impact on patients in both diagnosis and risk prediction.

CCTA has been used to demonstrate the differing plaque burdens and morphologies in PLWHIV. The specific difference of higher burdens of non-calcified plaque compared to non-HIV populations is suggestive of the unique metabolic and inflammatory phenotype that PLWHIV display. Early investigation into the effects of statin therapy suggest that these medications may reduce coronary plaque. Future goals of CCTA should focus on individualised risk stratification for PLWHIV as traditional risk calculators do not perform well in this unique group. Although the 'hybrid metabolic syndrome' that PLWHIV are exposed to seems a plausible mechanistic aetiology for driving excess CVD risk the data is discrepant. It may be that the presence of traditional risk factors has a synergistic effect with HIV on the development of atherosclerosis.

The role for CACS in PLWHIV will continue to be defined over the coming years. Although the technology for the non-invasive detection of CVD has progressed since the

advent of CACS it remains a powerful risk modifier in non-HIV populations. The extent to which this can be extended to PLWHIV needs to be investigated with longitudinal studies.

Future study using the multiparametric data available from CT datasets should yield important information regarding accurate CVD risk prediction. The use of traditional CVD scoring systems may be inadequate in this population. The use of routine quantification of EAT and HS as surrogates for CVD risk should be explored further and may represent targets for both pharmacological intervention and lifestyle modification, even in the absence of coronary plaque.

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