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Review Article

T-cell activation and cardiovascular risk in adults with type 2 diabetes mellitus: A systematic review and meta-analysis

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ABSTRACT

Background: Chronic immune activation has been described in the development of cardiovascular diseases (CVDs) and in the pathogenesis of type 2 diabetes mellitus (T2D). However, the precise functional role of T-cells remains controversial. We therefore, assessed T-cell activation and cardiovascular risk in T2D.
Methods: The protocol was registered with PROSPERO [CRD42018099745]. We searched electronic databases and grey literature for eligible studies. The risk of bias and quality of evidence were assessed and the random-effects model was used in the meta-analysis.
Findings: Fifteen studies met the inclusion criteria. We report on increased T-cell activation in T2D and non-diabetics with CVD. Comorbidity of T2D and CVD (T2D + CVD) exacerbated T-cell activation. In addition, T2D + CVD comorbidity was associated with an increased CVD risk profile.
Conclusion: This meta-analysis suggests increased T-cell activation in T2D and nondiabetics with CVD. Moreover, an increased cardiovascular risk in patients with T2D which is exacerbated in T2D and CVD co-morbidity.

1. Background

The burden of non-communicable diseases (NCDs) has drastically increased in both developing and developed countries [1]. This has led to a significant reduction in life expectancy and an increased strain on national healthcare budgets worldwide [2]. Globally, NCDs are the leading cause of death and account for up to 70% of all-cause mortality [1]. The global prevalence of type 2 diabetes mellitus (T2D), which is one of the major contributing factors to NCDs has significantly increased in the past three decades [3]. This has been attributed to sedentary lifestyle, rapid urbanisation and modernisation [3,4]. T2D is a low-grade chronic inflammatory condition that is characterised by hyperglycaemia, insulin resistance and chronic T-cell activation [5,6]. These consequences are consistent with immune activation that may lead to immune dysfunction and increased risk of cardiovascular diseases (CVDs) [7,8]. The latter is known to be the leading cause of death in individuals with diabetes [9], hence the need to unravel pathophysiological mechanisms such as the role of T-cell activation in a hyperglycaemic state to better understand and prevent NCDs.

The role of activated T-cells in mediating inflammation and altering myocardial function has been previously described. Whereby, activated $CD4^+$ T-cells were shown to promote myocardial ischaemia-reperfusion injury in mice [10]. Increased levels of pro-inflammatory T-helper (Th) subsets have been implicated in the development of coronary atherosclerotic heart disease (CHD) [11,12], carotid atherosclerosis (CA) [13] and coronary artery disease (CAD) [14] in individuals with T2D. Moreover, a significant reduction in the number of regulatory T-cells (Tregs) and Treg/Th1 ratios have been described in individuals with T2D and CHD [11].

Currently, it is hypothesised that chronic hyperglycaemia dysregulates T-cell function. However, the underlying mechanisms remain controversial, with contradictory findings of both elevated [15] and decreased [16] levels of T-cell activation reported in individuals with obesity and T2D. Others have demonstrated reduced frequency of Tregs

Abbreviations: CVDs, cardiovascular diseases; Th, T helper cells; T2D, type 2 diabetes mellitus

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in T2D and thus loss of the natural regulatory mechanisms mediated by T-cells [11,17]. This suggests that contradictory findings regarding T-cell function in T2D exist, and it remains unclear whether they are dysfunctional or highly activated in a disease state. Although numerous studies reported on T-cell function in T2D [6,15,16], to date, available evidence has not been systematically reviewed to better inform on both T-cell activation and cardiovascular risk in T2D. Therefore, this systematic review was conducted to assess available literature on the impact of T-cell activation in T2D and whether their activation state has any association with the risk of developing CVD. Furthermore, we assessed whether the degree of T-cell activation is unique to individuals with both T2D and CVDs or independently associated with those without T2D but presenting with CVDs.

2. Methods

This systematic review was prepared in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines [18]. A detailed checklist for this systematic review and meta-analysis is provided as PRISMA checklist (Supplementary file 1). The systematic review protocol was registered with the international prospective register of a systematic review (PROSPERO), registration number: CRD42018099745 and has been published [19].

2.1. Search strategy

A comprehensive search was conducted on the Cochrane Library, Embase and PubMed electronic databases from inception up to 20 October 2019 as previously described [19]. Briefly, two independent reviewers (TMN and VM) searched for relevant articles and a third reviewer (BBN) was consulted in cases of disagreements. Two search strategies were independently applied to identify relevant studies. The primary search strategy was on T-cell activation in individuals with T2D and CVDs (concept 1). Whilst the secondary search strategy was used to retrieve studies reporting on T-cell activation in nondiabetics with CVDs (concept 2). The search strategies were adapted to each database using keywords and medical subjects heading (MeSH) terms such as “Type 2 diabetes mellitus”, “hyperglycaemia”, “inflammation”, “CVDs”, “T-cell activation and exhaustion” and their respective synonyms and associated words/phrases. No language restrictions were applied. The study selection process was independently carried out by two reviewers (TMN and BBN). In cases of disagreements, PVD was consulted for arbitration. We used the Mendeley reference manager version 1.1.18 (Elsevier, Amsterdam, Netherlands) to identify and remove study duplicates.

2.2. Inclusion criteria

The systematic review and meta-analysis included studies reporting on T-cell function in adults (> 18 years) with CVDs and T2D. We excluded animal studies since we wanted to focus on human subjects. Other exclusions included books, letters, case reports, and reviews. Furthermore, we excluded studies that included participants using steatogenic medications or drugs that interfere with the immune system and patients with a known history of haematological malignancy.

2.3. Data extraction and quality assessment

The data extraction, synthesis and quality assessment of included studies were carried out as previously described [19]. Briefly, the extracted data items included; names of the authors, publication year, study design, study size, age, gender, types of CVD and main findings of each study. The risk of bias on the included studies was independently assessed by two reviewers (TMN and VM) and a third reviewer (PVD), was consulted in instances of disagreements using the modified Downs and Black checklist, which is suitable for both randomised and non-randomised studies [20]. Furthermore, the quality of evidence across the selected studies was assessed by two independent reviewers (TMN and VM) using the Grading of Recommendations Assessment Development and Evaluation (GRADE) approach [21].

2.4. Statistical analysis

The mean and standard deviation was extracted or calculated using *Hozo* et al.'s method for each continuous effect measure. Pearson's chi-squared test (Chi^2) and Higgin's I^2 statistics were used for the test for statistical heterogeneity. A random-effects model was used to generate pooled effect estimates when substantial heterogeneity existed ($I^2 > 50\%$). Effect sizes were interpreted according to Cohen's d method whereby a standardised mean difference of 0.2, 0.5 and 0.8 was equated to small, medium and large, respectively [22]. Moreover, a p-value < .05 was considered statistically significant and interrater reliability was assessed for both the included studies and risk of bias using Cohen's kappa. A kappa value of < 0.00 was interpreted as a poor strength of agreement, 0.00–0.20 as slight agreement, 0.21–0.40 as fair agreement, 0.41–0.60 as moderate agreement, 0.61–0.80 as substantial agreement and 0.81–1.00 as perfect agreement [23].

3. Results

3.1. Selected studies

A total of 151 studies were identified and screened for eligibility. A total of fifteen studies ($n = 15$) met the inclusion criteria (Fig. 1). Of these, 10 studies reported on T-cell activation in individuals with T2D whilst the remaining 5 reported on T-cell function in nondiabetics with CVD (overall agreement 91.53%, kappa = 0.75). The primary search strategy identified a total 76 studies, of which 66 studies were excluded due to no full-texts availability ($n = 13$) and presented no clear study design ($n = 9$). The majority of the studies ($n = 44$) were excluded because they were not relevant to the topic of interest. There were only 10 studies, published between 2011 and 2019, that met the inclusion criteria and 9 of these were included in the quantitative analysis. On the other hand, the secondary CVD search strategy (concept 2) retrieved 75 studies and a total of 62 studies were excluded because they were not relevant to the topic of interest, 4 were reviews and the other 4 were due to study design which contained no suitable controls [24,25]. Therefore, a total of 5 studies published between 2011 and 2014 fulfilled the inclusion criteria on T-cell activation in CVD and were included in this review. Of these studies, only 3 were included in the quantitative analysis.

3.2. Study characteristics

All included studies were published in peer-reviewed journals and characteristics of included participants are shown in Tables 1 and 2. Briefly, this study comprised of a total of 2744 participants with a mean age of 59.77 ± 13.60 years and a male/female gender ratio of 3.5. The included studies comprised of 6 prospective cohort studies [13,26–29] and 9 cross-sectional studies [11,12,14,30–35]. In total, 1062 individuals had T2D, 321 were nondiabetics with CVD and 1361 were healthy controls. In addition, 118 (11%) with T2D were on treatment and 944 (89%) were not specified, while 543 (51%) had T2D and 519 (49%) had both T2D and CVDs (T2D + CVD). The CVDs were all grouped into macrovascular complications and included a total of 840 individuals of which 304 had acute coronary syndrome (ACS) [26,29,30,33,34], 48 had atherosclerotic macrovascular complication (AS) [30], 30 had CA [13], 282 had CAD [14,31,34,35], 83 had CHD [12] and 93 had unspecified CVDs [27,28,32].

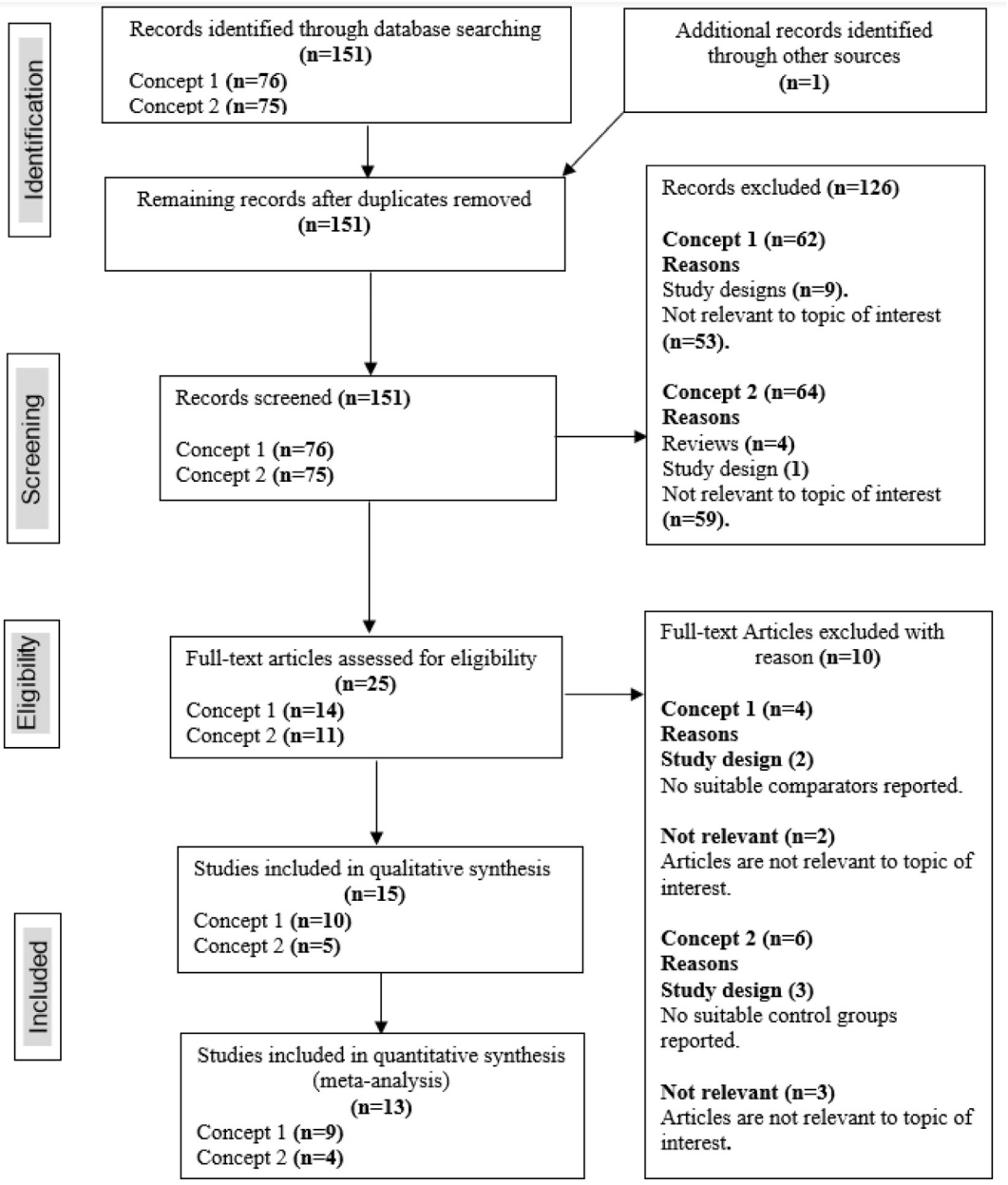

Fig. 1. Flow chart of study selection procedures.

3.3. Risk of bias assessment

The risk of bias for each study was assessed using the modified Downs and Black checklist [20]. The median score range of included studies was 12 (8–18) (Supplementary file 2). Seven of the studies were scored as fair (13–17 points) [14,26–29,32] and the rest poor (< 13 points) [11–13,30,31,33–35]. All the studies had low risk of reporting bias with a median of 6 (5–10) out of the possible score of 10 (overall agreement 83.89%, kappa = 0.68). The studies also had a relatively low risk of internal validity bias with a median of 3 (3–5) out of the possible score of 7 (overall agreement 75.08%, kappa = 0.50). All studies performed poor on the external validity (except 1 study) and selection bias domains with each a median of 0 (0–3) out of the possible score of 3 (overall agreement 77.04%, kappa = 0.74) and 1 (0–3) out of the possible score of 6 (overall agreement 88.34%, kappa = 0.77), respectively. The funnel plots showed perfect symmetry on included studies (Fig. 1S).

3.4. Data synthesis of included studies

All included studies showed increased T-cell function in T2D, whereby 8 studies reported increased T-cell activation in T2D when compared to normoglycaemic controls [11,13,14,26,27,30–32]. Whilst 4 studies reported increased T-cell activation in nondiabetics with CVD when compared to controls [26,29,33,35]. Moreover, 5 studies reported increased pro-inflammatory T-helper subsets in individuals with T2D + CVD compared to controls [11,13,28,31,32], while 1 study reported on a reduced number of immunosuppressive Tregs [11]. Interestingly, 2 studies reported on a reduced number of anti-inflammatory T-helper subset and their cytokines [13,31] in both T2D and T2D + CVD groups compared to the controls.

3.4.1. Reported glucose metabolic profiles

Of the 10 included studies, 6 reported on glucose metabolic profiles between different groups [11,12,14,26,28,31]. Overall, the lowest mean body mass index (BMI) was reported in the control group

Table 1
Characteristics of included studies on T-cell activation in individuals with T2D and CVD (n = 10).

Study	Country	Study design	Study size	Male, n (%)	Age (Years)	Risk of bias	Reported measures of immune activation/T-cell activation	Main findings
Rattik et al. (2019)[28]	Sweden	Cohort study	153 participants. (55 T2D; 54 T2D + CVD and 44 controls)	103 (67%)	73.71 ± 2.06	Fair	Effector memory T-cells and Tregs	Effector memory T-cells are potential biomarker for CVDs in individuals with T2D. Moreover, there was no difference in the proportion of Tregs between T2D and (T2D + CVD) group.
Gong et al. (2016)[14]	China	Cross sectional	359 participants. (76 T2D; 158 T2D + CAD and 125 controls)	293 (56.9%)	62.91 ± 11	Fair	IL-17, IL-22, IL-9 and IL-27	Serum IL-22 levels are elevated in T2D, coronary CAD and T2D-CAD comorbidity than controls. Furthermore, IL-22 protects endothelial cells from glucose and lysophosphatidylcholine-induced injury.
Wang et al. (2016)[13]	China	Cohort study	60 participants. (20 T2D; 30 T2D + CA and 10 controls)	23 (38.3%)	51.85 ± 17.62	Poor	Th1, Th2, Th17, INF- γ, IL-4 and IL-17	Angiotensin II promotes the development of CA in T2D patients via regulating the T-cells activities.
Eldor et al. (2015)[27]	Israel	Cohort study	89 participants. (23 T2D; 26 T2D + CVD and 40 controls)	Incalculable	Incalculable	Fair	%CD247 and CRP	The expression of CD247 in significantly reduced in individuals with T2D and is associated with the development of CVDs. Thus, CD247 is a potential diagnostic and prognostic marker for detecting disease progression and severity.
Olson et al. (2015)[32]	USA	Cross sectional	889 participants. (141 T2D; 13 T2D + CVD and 735 controls)	Incalculable	Incalculable	Fair	Th1, Th2, %CD4 naïve and %CD4 memory-cells	T-cells are involved in the chronic immune activation and suppression observed in T2D.
Zhao et al. (2014)[12]	China	Cross sectional	109 participants. (42 T2D and 67 T2D + CHD)	58 (53.23)	59.67 ± 13.76	Poor	hs-CRP, Th1, Th17, Th22 and percentage expression of CD4+ T-cells	Increased peripheral proinflammatory Th subsets contribute to the increased prevalence of diabetic cardiovasculopathy. Elevated Th subsets are also associated with increased CRP levels.
Madhumitha et al. (2014)[31]	India	Cross sectional	142 participants. (60 T2DM; 21 T2DM + CAD and 61 controls)	Not reported	48.29 ± 16.46	Fair	Th1 cytokines (IL-2, IL-12 and INF-γ) and Th2 cytokines (IL-4, IL-5 and IL-13)	The transition from T2D to T2D-CAD co-morbidity, is associated with strong downregulation of Th2 cytokines and upregulation of Th1 responses.
Shi et al. (2013)[30]	China	Cross sectional	173 participants. (42 T2DM; 48 T2DM + AS; 35 T2DM + ACS and 48 controls)	No reported	52.56 ± 10.25	Poor	$CD4^+CD28^-$, $CD4^+CD28^-PD\text{-}1^+$ and INF- γ	The upregulation of PD-1 is closely associated with the severity of diabetic atherosclerotic macrovascular diseases.
Mahmoud et al. (2013)[11]	Kuwait	Cross sectional	70 participants. (24 T2D; 16 T2D + CHD and 30 controls)	44 (62.9%)	50.41 ± 1.95	Poor	Th1, Th17, Treg and Expression of $CD4^+IFN\gamma^+$, $CD4^+TNF\text{-}\alpha^+$, $CD4^+IL\text{-}8^+$, $CD4^+IL\text{-}6^+$, $CD4^+IL\text{-}1??^+$ and $CD4^+IL\text{-}17^+$ T-cells	Hyperglycaemia and dyslipidaemia are associated with an increased inflammatory cytokine expression, suggesting the involvement of T cells in the development of T2D and CHD as its complication.
Giubilato et al. (2011)[26]	Italy	Cohort study	171 participants. (60 T2D; 51 T2D + ACS and 60 controls)	213 (74.5%)	62.44 ± 9.61	Fair	%$CD4^+$ T-cells, %$CD4^+CD28^-$ expression and hs-CRP	There is a higher prevalence of $CD4^+CD28^-$ T-cells in individuals with T2D. Furthermore, the T-cell subset is associated with poor hyperglycaemic control and the occurrence of first cardiovascular event

Abbreviations: ACS – acute coronary syndrome; AS – atherosclerotic macrovascular complication; CA – carotid atherosclerosis; CAD – coronary artery disease; CHD – Coronary heart disease; CVD – cardiovascular disease; hs-CRP – highly sensitive C-reactive Protein; IL – Interleukin; INF-γ – Interferon gamma; PD-1 – Programmed Cell death-1; Th – T helper; TNF- α – Tumour necrosis factor-alpha; Treg – regulatory T-cells; T2D – Type 2 diabetes mellitus.

Table 2
Characteristics of included studies on T-cell activation in nondiabetics with CVD (n = 5).

Study	Country	Study design	Study size	Male, n (%)	Age (years)	Risk of bias	Reported measures of immune activation/t-cell activation	Main findings
Emoto et al. (2014)[35]	Japan	Cross sectional	140 participants. (73 CAD and 67 controls)	118 (84.3%)	59.75 ± 9.49	Poor	%$CD4^+$, %$CD4^+CD28^-$ expression and hs-CRP	Individuals with CAD had increased percentage expression of $CD4^+CD428^-$ T-cells but decreased Tregs as well as Treg/non-Treg $CD4^+$ T-cells ratio when compared to controls. Moreover, CAD patients had increased hs-CRP levels compared to controls.
Flego et al. (2014)[29]	Italy	Cohort study	70 participants. (35 ACS and 35controls)	47 (67.1%)	63.5 ± 10.98	Fair	%$CD4^+$, %$CD4^+CD28^-$ expression and hs-CRP	Individuals with ACS had increased frequency of $CD4^+CD28^-$ T-cells compared to controls. Moreover, the inhibitory effect of CD31 on TCR signalling of $CD4^+$ and $CD4^+CD28^-$ T cells was reduced in ACS patients compared to controls.
Teo et al. (2013)[34]	Brazil	Cross sectional	66 participants. (20 ACS, 30 CAD and 16 controls)	47 (71.2%)	60.88 ± 8.82	Poor	$\%CD4^+CD28^-$ expression, IFN-γ, TNF-α and CRP	There was no difference in the frequency of $CD4^+CD28^-$ T-cells between all groups. However, $CD4^+CD28^-$ T-cells were the main source of pro-inflammatory cytokines in CAD.
Dumitriu et al. (2012)[33]	England	Cross sectional	78 participants. (48 ACS and 30 controls)	50 (64.1%)	61.98 ± 13.33	Poor	$\%CD4^+CD28^-$ expression, IFN-γ, and TNF-α	The frequency of $CD4^+CD28^-$ T-cells was higher in ACS compared to controls. In addition, the $CD4^+CD28^-$ T-cells expressed higher levels of the alternative co-stimulatory receptors (OX40 and 4-1BB) when compared to classical $CD4^+CD28^+$ T-cells.
Giubilato et al. (2011)[26]	Italy	Cohort study	175 participants. (115 ACS and 60 controls)	136 (77.7%)	60.97 ± 9.45	Fair	%$CD4^+$, %$CD4^+CD28^-$ expression and hs-CRP	ACS patients had increased prevalence of $CD4^+CD28^-$ T-cells when compared to controls.

Abbreviations: ACS – acute coronary syndrome; CAD – coronary artery disease; hs-CRP – highly sensitive C-reactive Protein; INF-γ – Interferon gamma; Th – T helper; TNF-α – Tumour necrosis factor-alpha; Treg – regulatory T-cells.

(24.05 ± 3.32) when compared to both T2D (26.60 ± 4.02) and T2D + CVD (25.93 ± 3.35) groups. A meta-analysis between the T2D and the control group showed significant heterogeneity between included studies (Chi^2 = 12.31, I^2 = 76%, P = .06). The included studies reported on significantly higher BMI in individuals with T2D when compared to the control group ([MD = 2.25, 95% CI (1.41; 3.09), p < .00001]) (Fig. 2S). Relevant to HbA1c level, which is a measure of metabolic state of diabetes for the last three months, only 4 studies reported on increased HbA1c levels in the T2D compared to the control group ([MD = 2.06, 95% CI (1.42; 2.70), p < .00001]) [11,14,28,31]. However, there were significant levels of unexplained statistical heterogeneity amongst these 4 studies (Chi^2 = 113.25, I^2 = 97%, p < .00001) (Fig. 3S).

3.4.2. Reported effect measure of T-cell activation

Increased expression of the rare pro-atherogenic $CD4^+CD28^-$ T-cells was reported by 2 studies and was shown to be higher in T2D compared to controls, with a mean percentage of 7.85 ± 0.88 and 1.82 ± 0.45, respectively [26,30]. The pooled effect estimates showed a large effect size in percentage expression of $CD4^+CD28^-$ T-cells in individuals with T2D when compared to healthy controls ([MD = 4.02, 95% CI (−0.62; 8.65), p = .09) (Fig. 2A). Moreover, T2D and CVD comorbidity was significantly associated with increased circulating $CD4^+CD28^-$ T-cells, as the mean increased to 21.34 ± 12.47 compared to the T2D group ([MD = 11.44, 95% CI (8.27; 14.62), p < .00001]). However, substantial level of heterogeneity was present in these studies (Chi^2 = 2.03 and I^2 = 51%, p < .00001) (Fig. 2B). On the other hand, there was increased level of $CD4^+CD28^-$ T-cells in nondiabetic with CVD when compared to controls. ([MD = 2.16, 95% CI (0.23; 4.08), p = .03], Chi^2 = 48.85 and I^2 = 96%, p < .00001) (Fig. 2C). Interestingly, although this pooled estimate also revealed significant difference between the nondiabetics with CVD and control group, the overall mean difference (Z = 2.02) was of small effect size (0.2) when compared to that of T2D (Z = 7.06), medium effect size (0.7).

3.4.3. Reported effect measures of cardiovascular risks

3.4.3.1. Overall pooled estimates for cardiovascular risk. Pooled standard mean differences showed reduced CVD risk in controls compared to individuals with T2D ([SMD = −0.34, 95% CI (−0.78; 0.10), p = .13], Chi^2 = 466.36, I^2 = 96%, p < .00001) (Fig. 3). Notably, one of the included studies [11] showed significantly different study-level outcome in CVD risk profile. When the data from this study were omitted, there was a small effect size between T2D group and healthy controls ([SMD = 0.03, 95% CI (−0.30; 0.35), p = .87], Chi^2 = 191.96, I^2 = 92%, p < .00001). Moreover, pooled estimates showed an insignificant increased odds risk of CVD in individuals with T2D when compared to controls ([OR = 0.94, 95% CI (0.45; 1.97), p = .87], Chi^2 = 22.47, I^2 = 78%, p = .0004) (Fig. 5A). As expected, odds risk of CVD was higher in nondiabetics with CVD group when compared to controls ([OR = 2.33, 95% CI (1.75; 3.09), p < .00001)], Chi^2 = 43.01, I^2 = 88%, p < .00001 (Fig. 5B). However, due to substantial level of statistical heterogeneity in these pooled estimates in Figs. 3 and 4, a subgroup analysis based on the reported effect measure of cardiovascular risk was conducted.

3.4.3.2. Body mass index. Overall, data from the included 5 studies showed a lower BMI mean in T2D + CVD group (25.93 ± 3.35) when compared to the T2D group (26.60 ± 4.02) [11,12,14,28,31].

3.4.3.3. Total cholesterol. Five of the included studies [11,13,14,26,31] reported no significant difference in total cholesterol levels between the T2D group and controls ([SMD = 0.07, 95% CI (−0.74; 0.88), p = .87]) (Fig. 3). However, there was substantial level of statistical heterogeneity in these studies (Chi^2 = 69.28 and I^2 = 94%, p < .00001). In addition, there was no difference in total cholesterol

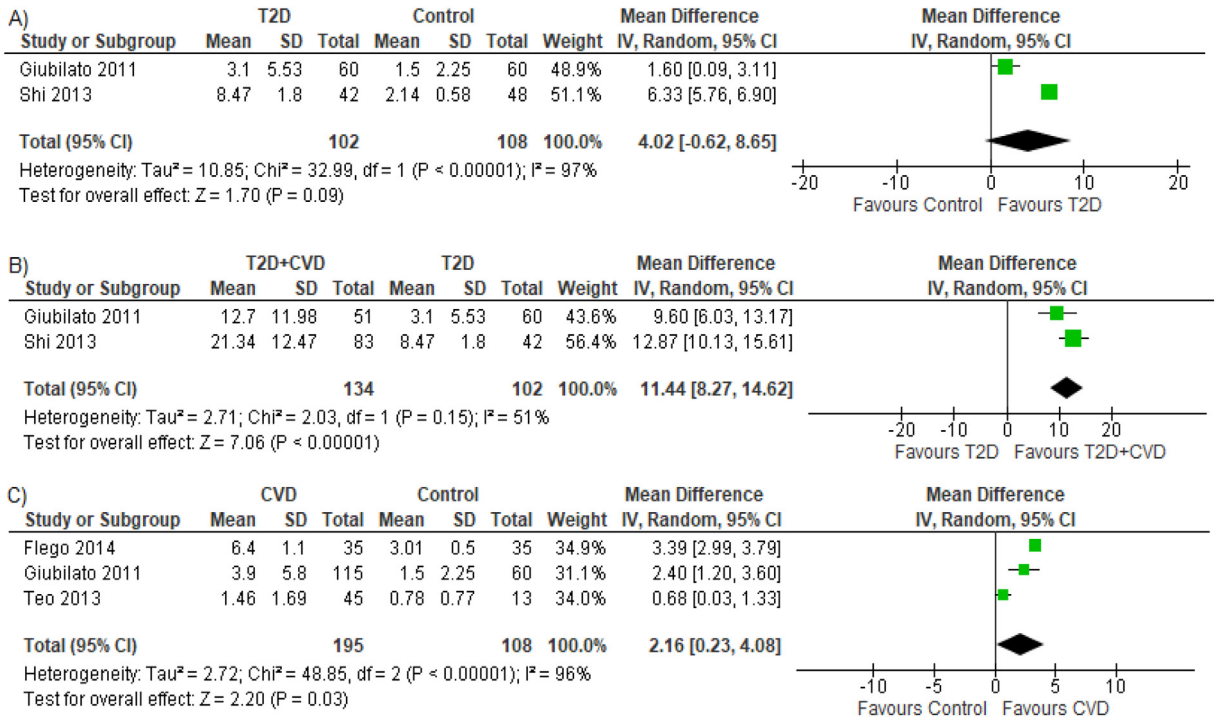

Fig. 2. T-cell activation measured by the expression of $CD4^{+}CD28^{-}$ T-cells in (A) T2D compared to controls; (B) in individuals with T2D + CVD compared to T2D; (C) in nondiabetics with CVD compared to controls.

levels in T2D and T2D + CVD groups ([SMD = −0.03, 95% CI (−1.01; 0.96), p = .96], Chi^2 = 84.16, I^2 = 95%, p < .00001) (Fig. 4). Similarly, there was no significant difference in total cholesterol levels between nondiabetics with CVD and healthy controls ([SMD = −0.17, 95% CI (−0.50; 0.15), p = .30], Chi^2 = 3.36, I^2 = 41%, p = .19) (Fig. 4S).

3.4.3.4. High-density lipoprotein. Pooled estimates from 6 studies [11,13,14,26,28,31] revealed decreased high-density lipoprotein (HDL) levels in individuals with T2D when compared to controls ([SMD = −0.86, 95% CI (−1.65; −0.07), p = .03]). However, there were substantial levels of statistical heterogeneity in these studies (Chi^2 = 94.68 and I^2 = 95%, p < .00001) (Fig. 3). Data from 7 included studies [11–14,26,28,31] showed a large effect size difference in HDL levels between the T2D (1.22 ± 0.45) and T2D + CVD (1.25 ± 0.51) groups ([SMD = −0.90, 95% CI (−1.82; 0.03), p = .06] Chi^2 = 141.85, I^2 = 96%, p < .00001) (Fig. 4). In addition, there was a medium effect size difference in HDL levels of nondiabetics with CVD and control group ([SMD = −0.53, 95% CI (−1.14; 0.07), p = .08] Chi^2 = 25.77, I^2 = 88%, p < .00001) (Fig. 4S).

3.4.3.5. Low-density lipoprotein. A total of 6 studies reported on decreased levels of low-density lipoprotein (LDL) in T2D when compared to the control group ([SMD = −1.18, 95% CI (−2.06; −0.30), p = .009]). The included studies showed substantial levels of statistical heterogeneity (Chi^2 = 117.32 and I^2 = 96%, p < .00001) (Fig. 3). Notably, T2D + CVD group was significantly associated with increased LDL levels compared to T2D group ([SMD = 0.90, 95% CI (0.30; 1.50), p = .0003] Chi^2 = 51.44, I^2 = 90%, p < .00001) (Fig. 4). On the other hand, there was no significant difference in LDL levels between nondiabetics with CVD and control group ([SMD = −0.20, 95% CI (−0.56; 0.17), p = .29] Chi^2 = 9.65, I^2 = 69%, p = .02) (Fig. 4S).

3.4.3.6. C-reactive protein levels. A total of 621 participants from 6 studies were included in this analysis [12,26,27,29,34,35] and the results revealed that individuals with T2D + CVD had higher CRP mean levels (12.38 ± 17.22) when compared to both nondiabetics with CVD (5.75 ± 16.30) and controls (1.15 ± 1.14). Notably, pooled estimates showed a significant increase in CRP levels of nondiabetics with CVD when compared to controls ([SMD = 0.35, 95% CI (0.15; 0.54), p = .0005] Chi^2 = 1.45, I^2 = 0%, p = .69) (Fig. 4S).

3.4.3.7. Hypertension. Three studies reported an increased prevalence of hypertension in individuals with T2D + CVD (mean ratio 0.75), compared to T2D (mean ratio 0.63) and healthy controls (mean ratio 0.55) [14,26,28]. Individuals with T2D showed no association with the prevalence of hypertension when compared to the control group ([OR = 1.34, 95% CI (0.90; 1.99), p = .15]). There was no heterogeneity in the included studies (Chi^2 = 1.62 and I^2 = 0%, p = .45) (Fig. 5A). Hypertension was associated with the presence of known CVD and T2D (OR = 1.90, 95% CI (1.24; 2.91), p = .003], Chi^2 = 2.09 and I^2 = 4%, p = .35) (Fig. 5S). As expected, the prevalence of hypertension was associated with known cases of CVD when compared to controls (OR = 2.74, 95% CI (1.87; 4.02), p < .00001], Chi^2 = 44.34 and I^2 = 95%, p < .00001) (Fig. 5B).

3.4.3.8. Smoking. Three studies reported on smoking as a risk factor for CVDs [13,14,26]. There was no association between smoking in T2D and controls ([OR = 0.60, 95% CI (0.16; 2.31), p = .46]) (Fig. 5A). However, a substantial level of heterogeneity was present in these studies (Chi^2 = 8.87 and I^2 = 77%, p = .01). Similarly, there was no association between smoking in T2D and T2D + CVD groups ([OR = 1.91, 95% CI (0.57; 6.42), p = .30], Chi^2 = 9.83 and I^2 = 80%, p = .007) (Fig. 5S). Whereas smoking was associated with CVDs in nondiabetics when compared to controls (OR = 1.90, 95% CI (1.24; 2.91), p = .003], Chi^2 = 1.49 and I^2 = 0%, p = .47) (Fig. 5B). The main findings of this meta-analysis are presented in the summary of findings table (Table 3).

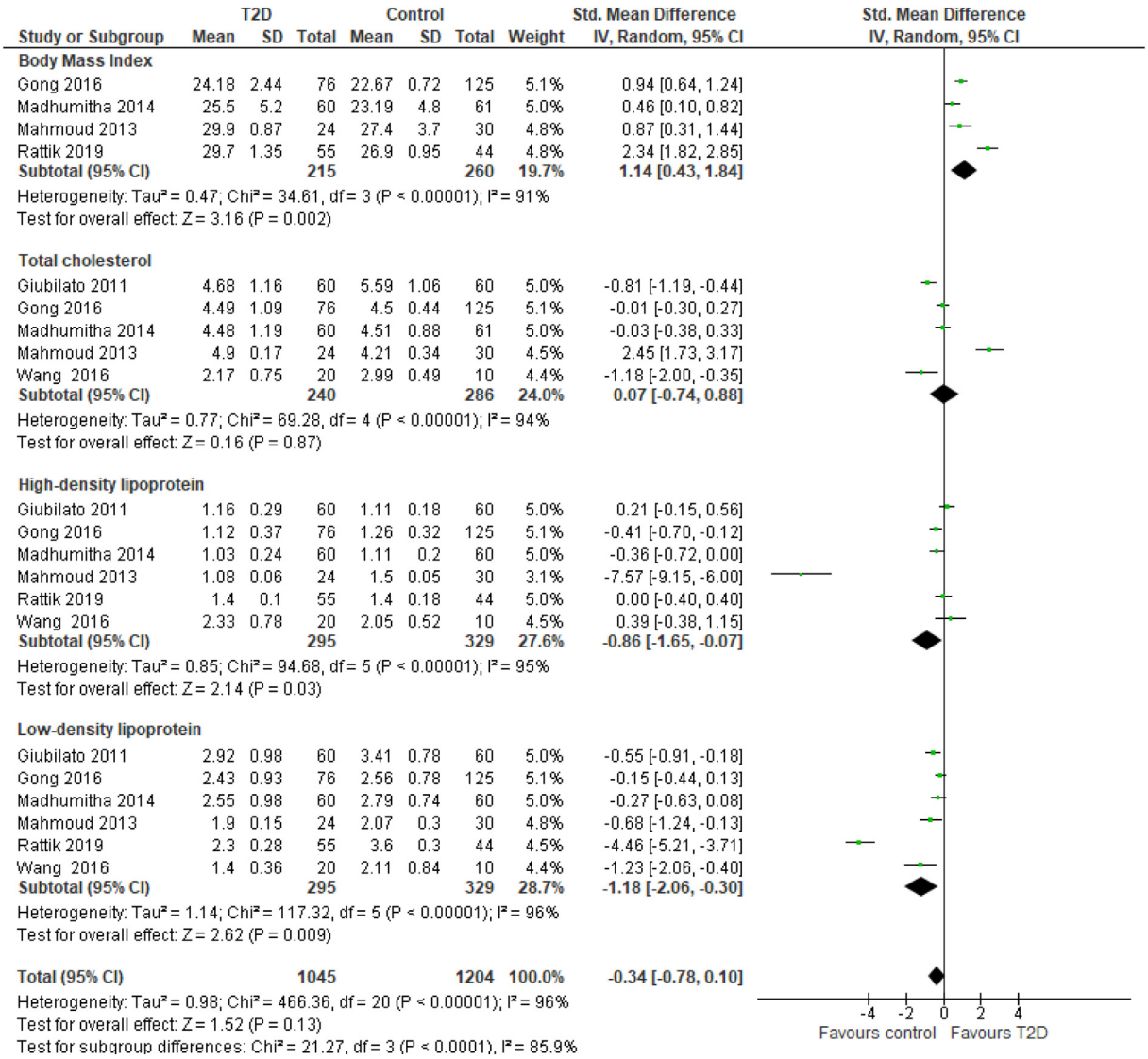

Fig. 3. Pooled estimates of cardiovascular risk in T2D compared to controls.

3.5. A narrative synthesis of included studies

3.5.1. Expression of Th subsets

Four of the included studies reported differences in Th subsets between T2D and control groups [11–13,32]. Of these, 1 study demonstrated no significant differences in the Th subsets between the T2D group and the control [32]. However, 2 reported increased expression of pro-inflammatory Th1 in T2D + CVD group when compared to T2D [12,13]. In addition, 2 studies revealed an upregulated expression of Th17 in individuals with T2D when compared to controls [11,13]. Moreover, 3 of these studies associated the presence of CVD in T2D with a further increase in Th17 expression [11–13].

One study reported a decreased expression of anti-inflammatory Th2 subset in T2D when compared to the control group [13]. The presence of a CVD in T2D further decreased the expression of Th2 [13]. The same study reported a similar pattern with Tregs whereby their expression was decreased in T2D when compared to controls. Furthermore, the presence of a CVD in T2D was associated with a further decrease in Tregs expression [13]. On the other hand, increased frequency of $CD4^+CD28^-$ T-cells was reported in individuals with CVD compared to controls in 4 of the included studies [26,29,33,35]. However, 1 study reported no difference in the expression of $CD4^+CD28^-$ T-cells between the CVD and control groups [34]. A meta-analysis could not be performed on T-cell subsets due to lack of data for statistical analysis.

3.5.2. Pro-inflammatory cytokines

Increased circulating pro-inflammatory cytokines in T2D were reported in 5 of the 9 included studies [11,13,14,30,31]. Of these studies, 4 reported on increased interferon gamma (INF-γ) levels in T2D when compared to controls [13,14,30,31]. Interferon-gamma (INF-γ) is a pro-inflammatory signature cytokine for Th1 [36]. Furthermore, these studies associated the presence of CVD in T2D with a further increase in INF-γ levels. Although 3 studies reported on differences in interleukin (IL)-17 (a signature cytokine for Th17), only 2 reported increased expression of IL-17 in T2D when compared to controls [11,14]. The other study showed decreased IL-17 in T2D when compared to controls [13]. Nevertheless, all studies associated the presence of CVD in T2D with increased IL-17 levels when compared to both controls and T2D groups [11,13,14]. Two of the included studies reporting on T-cell activation in CVD, showed increased secretion of pro-inflammatory IFN-γ and TNF-α cytokines in individuals with CVD compared to controls [33,34]. A meta-analysis could not be performed on pro-inflammatory cytokines due to lack of data for statistical analysis hence these effect measures are reported narratively.

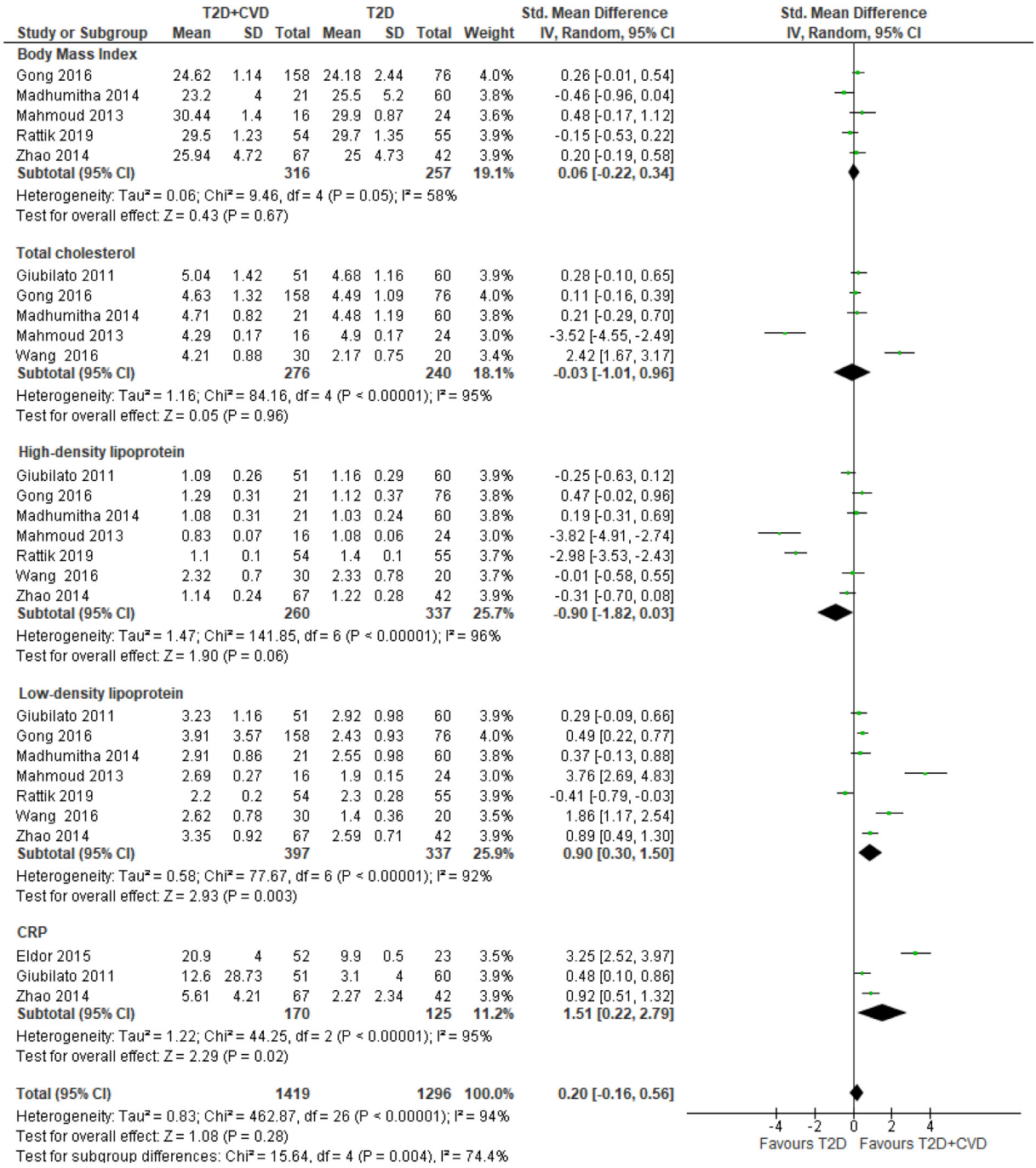

Fig. 4. Pooled estimates of cardiovascular risk in T2D compared T2D + CVD.

3.5.3. Anti-inflammatory cytokines

Two studies reported on Th_2 anti-inflammatory cytokines [13,31]. Increased levels of IL-4 were demonstrated in T2D compared to healthy controls, while Wang et al showed no significant difference between the 2 groups [13,31]. However, in 1 study, the presence of a CVD in T2D further decreased IL-4 levels when compared to both T2D and the control group [31]. No meta-analysis could be performed due to lack of data for statistical analysis.

4. Discussion

This systematic review aimed at assessing available literature describing the role of T-cell activation and other markers of inflammation in the development of CVDs in T2D. Majority of included studies showed increased T-cell activation in individuals with T2D when compared to the control group. Furthermore, synthesised data suggested that individuals with T2D are at higher risk of developing CVD albeit the data was from observational studies. It was also clear that none of the included studies measured T-cell exhaustion. Moreover, T-cell activation is increased in nondiabetics with CVD when compared to controls. Therefore, these findings suggest that increased T-cell activation is not unique to T2D but the degree of activation is exacerbated by the presence of T2D.

Increased Th_1 and Th_{17} subsets and loss of Tregs cells have been implicated in the pathogenesis of inflammatory disease [37–40]. Our synthesised data provides a comprehensive increased level of pro-inflammatory Th subsets in T2D, thus implicating increased inflammation and T-cell activation in the development of CVDs. Moreover, elevated

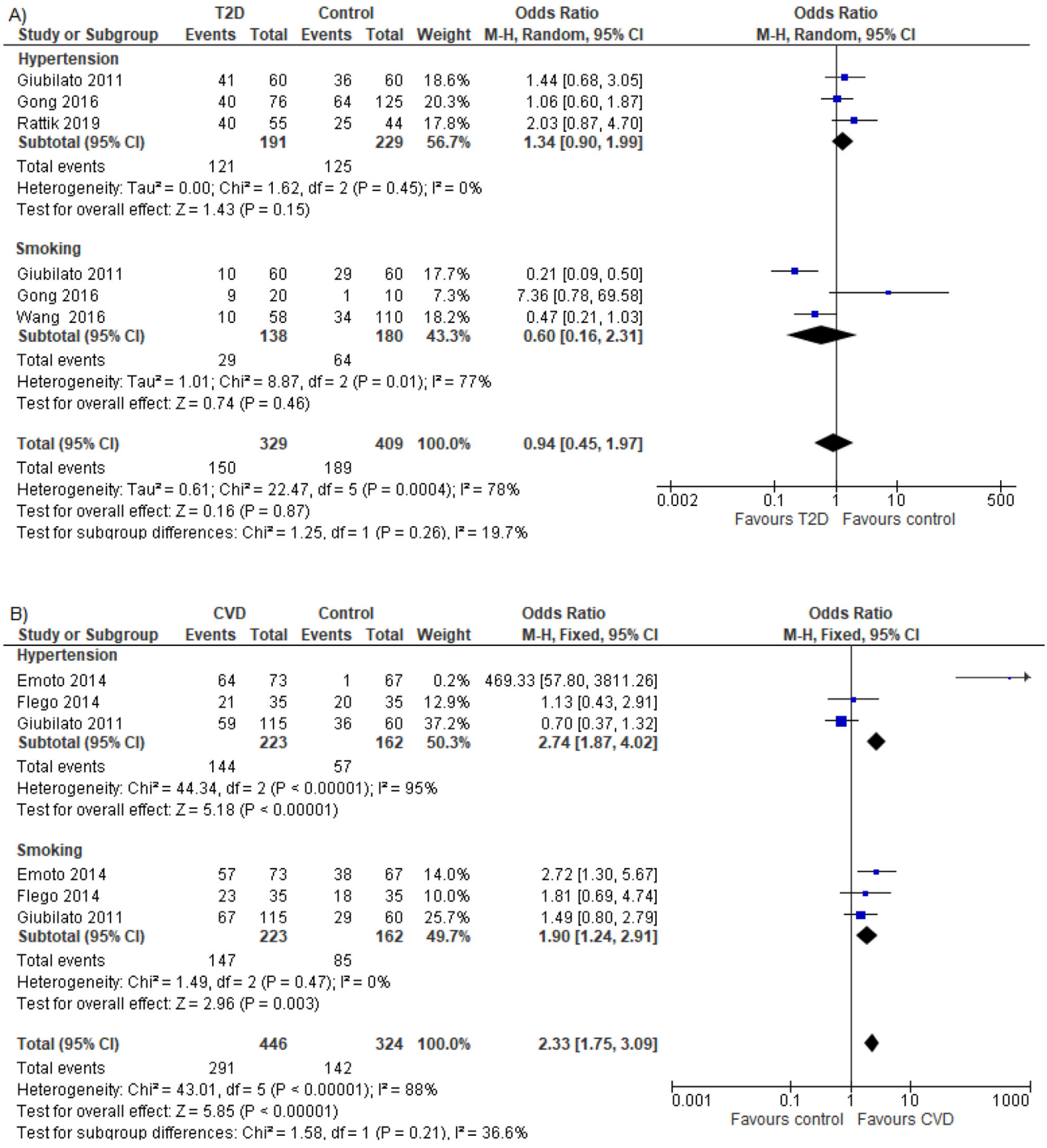

Fig. 5. The prevalence of cardiovascular risk factors in (A) T2D compared to healthy controls and in (B) nondiabetics with CVD compared to healthy controls.

pro-inflammatory cytokines and CRP levels reported in individuals with T2D further implicate chronic inflammation as a link between T2D and increased risk of developing CVD.

Tregs have been reported to have a protective role against the development of CVDs. In that context, low levels of circulating Tregs and Th_2 have been associated with increased risk of acute coronary events [41,42]. Data synthesised in this review showed lower levels of Th_2 anti-inflammatory cytokines and a reduction in the number of Tregs in individuals with T2D compared to controls. Therefore, exacerbated inflammation with T-cell activation and decreased T-cell immunosuppressive potential may be associated with the development of CVDs in T2D.

The $CD4^+CD28^-$ T-cells are a long-lived Th_1 subset that has both proatherogenic and plaque-destabilising properties [33]. However, unlike conventional Th_1 cells, these T-cells also express cytotoxic molecules (perforin and granzyme B) and are rarely found in healthy individuals [43]. Nonetheless, this subset has been implicated in the pathogenesis of various inflammatory disorders [33,44]. In chronic inflammation, the $CD4^+CD28^+$ T-cells lose the expression of CD28, a co-stimulatory marker and therefore making them insensitive to both suppression and apoptotic responses [44]. In addition, $CD4^+CD28^-$ T-cells release an abundant amount of pro-inflammatory cytokines and cytotoxic mediators which are responsible for tissue damage in the pathogenesis of inflammatory disorders such as CVD [33,43]. Data

Table 3
Summary of findings table.

T2D compared to control group

Patient or population: Adult individuals with T2D
Exposure: T2D
Comparison: Non-diabetic (control)

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with Control	Risk with T2D				
T-cell Activation Scale from: 1.5 to 8.47%	The mean t-cell activation was **1.82**%	The mean t-cell activation in the exposure group was 4.02% higher (0.62 lower to 8.65 higher)	–	210 (2 observational studies)	⨁⨁oo LOW	
Cardiovascular Disease Risk Measured using HDL Scale from: 1.4 to 2.33 mmol/l	The mean for HDL was **1.41** mmol/l	The mean for HDL in the exposure group 0.86 mmol/l lower (−1.65 lower to −0.07 lower)	–	525 (5 observational studies)	⨁⨁oo LOW	
T-cell exhaustion Not measured	See comment	See comment	NE	–	See comment	None of the included studies measured T-cell exhaustion

***The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval; **MD:** Mean difference; **OR:** Odds ratio; **NE:** Not estimable

GRADE Working Group grades of evidence
High certainty: We are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.
Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

synthesised in this systematic review showed a high prevalence of $CD4^+CD28^-$ T-cells and increased pro-inflammatory cytokine release, in individuals with T2D as well as nondiabetics with CVD. Interestingly, although both these groups reported increased T-cell activation when compared to their respective control groups, the effect size was greater in individuals with T2D compared to those without [22]. Nonetheless, it is evident that T-cell activation is exacerbated by the presence of T2D and is implicated in the development of CVDs in T2D.

It is well-established that individuals with T2D have a higher cardiovascular risk and mortality rate when compared to their non-diabetic counterparts and are disproportionately affected with CVDs [45]. Hypertension is a well-established cardiovascular risk factor present in two-thirds of individuals with T2D [46]. Moreover, the co-existence of hypertension and T2D increases the risk of developing CVDs by almost four-fold when compared to controls [47]. Our study showed a significant association between hypertension and CVDs. Dyslipidaemia, another major risk factor of CVDs that is characterised by changes in both quality and quantity of lipoproteins plays a significant role in the development of atherosclerosis [48]. In that context, high and low levels of LDL and HDL were demonstrated to be closely associated with T2D [48,49]. In accordance with this, data synthesised from this review showed dyslipidaemia as a characteristic feature of T2D, while the presence of CVD was significantly associated with an increased degree of dyslipidaemia. These findings support the notation that individuals with T2D are at a higher risk of developing CVD. Therefore, hypertension and lipid profiles (particularly HDL) may be used as good markers for cardiovascular risk stratification and potential therapeutic targets in CVDs.

To date, this is the first systematic review and meta-analysis that comprehensively assessed T-cell function in individuals with T2D and their association with increased risk of developing CVD. In addition, the evidence presented in this review indicates that T-cells may be a potential therapeutic target in the management of T2D, although these data were synthesised from observational studies. These findings pave the way for future studies to explore novel avenues in developing new drugs for both management and treatment of diabetes.

The limitations of the current systematic review include; a restricted number of studies investigating the role of T-cells in both T2D + CVDs. In addition, none of the included studies were from African regions, where there is increased urbanisation and risk of CVDs. Secondly, there was a high risk of bias in 8 of the included studies [11–13,30,31,33–35] and the cross-sectional nature of all the included studies was also a significant limitation. Thirdly, the included body of evidence was from observational studies and thus is of low quality. This consequence therefore lowered the certainty of associations between T-cell activation and cardiovascular risk in T2D. Further, randomised controlled trials studies with high-quality evidence and reduced risk of bias due to randomisation are needed to address this. Lastly, although several studies reported on the different anti-hyperglycaemic treatments used by individuals with T2D [11,26], there were insufficient study-level data to perform any sub-group analysis. Therefore, we could not ascertain the effect of anti-hyperglycaemic drugs on T-cell function and cardiovascular risk.

5. Conclusion

The evidence from the included studies showed that peripheral blood T-cells are activated in individuals with T2D or CVD. Moreover, there is increased cardiovascular risk in individuals with T2D. Notably, the transition from T2D to T2D + CVD co-morbidity is associated with exacerbated levels of T-cell activation and increased cardiovascular risk. This was indicated by increased levels of $CD4^+CD28^-$ T-cells, LDL, CRP and decreased HDL as well as the development of hypertension, leading to a poorer prognosis. In addition, increased T-cell activation in T2D is coupled with a decreased frequency of peripheral immunosuppressive Tregs, increased frequency of pro-inflammatory T-

helper subsets and cytokines, including enhanced expression of T-cell negative co-stimulatory molecules. Therefore, a possible approach to reduce the risk of developing CVD in T2D is by modulating T-cell activation, which could be effective in alleviating immune suppression or inflammation. Furthermore, the use of interventions that target and alter $CD4^+$ T-cell subpopulations in T2D could be beneficial in reducing the risk of developing CVD.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary files.

Declaration of Competing Interests

We declare no competing interests associated with this manuscript.

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Authors' contribution

TMN, PVD, VM and BBN conceptualised, designed and drafted this manuscript. All authors wrote and approved the final manuscript. TMN is the guarantor of the review.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.108313.

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