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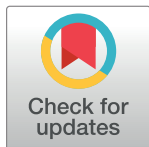
RESEARCH ARTICLE

Improvement of non-invasive tests of liver steatosis and fibrosis as indicators for non-alcoholic fatty liver disease in type 2 diabetes mellitus patients with elevated cardiovascular risk profile using the PPAR- α/γ agonist aleglitazar

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Data Availability Statement: There are legal restrictions to sharing a de-identified dataset as the AleCardio trial data are owned by a third-party organization (Hoffmann-La Roche). After the end of the study a Steering Committee was established as governing body for data management and to hold scientific responsibility. The steering committee can be reached by contacting Julius Clinical, a scientific CRO, which carried out the data

Abstract

Background

Peroxisome proliferator-activated receptor (PPAR) agonists may have favorable outcomes on non-alcoholic fatty liver disease. This study serves as proof of concept to evaluate whether dual PPAR- α/γ agonists improve non-invasive tests of liver steatosis and fibrosis.

Methods

This is a post-hoc analysis of a randomized, double-blind, placebo-controlled, multi-center trial comprising 7226 patients with type 2 diabetes mellitus and recent coronary artery disease randomized to receive aleglitazar, a PPAR- α/γ agonists, or placebo for two years. Main outcomes were change in non-invasive tests for liver steatosis and fibrosis: Liver Fat Score (LFS), Liver Accumulation Product (LAP), Fibrosis-4 (FIB-4), and NAFLD Fibrosis Score (NFS).

Results

LFS, LAP and FIB-4 decreased upon treatment, whereas scores in the placebo group remained the same or increased ($P < 0.001$). NFS responded differently but remained

management of the original trial. Contact: Julius Clinical, Zeist, the Netherlands, <https://www.juliusclinical.com/contact/>.

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consistently lower than placebo. In the treatment group more participants shifted to a lower FIB-4 and NFS category, or improved in respect to the LAP cut-off values compared to the placebo group ($P < 0.001$ for FIB-4 and LAP, $P < 0.004$ for NFS). LFS had a low discriminative power in this study.

Conclusion

This post-hoc analysis showed improvement of non-invasive tests of liver steatosis and fibrosis after starting dual PPAR- α/γ agonist treatment, adding to the evidence that this pathway has potential in non-alcoholic fatty liver disease treatment.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, and its incidence is continuously rising [1]. NAFLD is a spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), NASH-related fibrosis, and ultimately even cirrhosis and hepatocellular carcinoma (HCC). Progressive stages of NAFLD have been associated with an increased risk of cardiovascular events, and liver fibrosis is related to liver-related mortality and all-cause mortality [2–4]. The etiology of NAFLD is complex and multifactorial, including insulin resistance, hormone secretion from adipose tissue, oxidative stress, gut microbiome dysbiosis and imbalance of inflammatory cytokines [5,6]. Current treatment options are limited to lifestyle changes, with weight loss and physical exercise as the cornerstones of treatment. While weight loss with bariatric surgery causes sustained decrease of steatosis and inflammation scores, thereby being the most effective treatment at present, it is invasive and expensive [7]. Currently, there are no approved pharmacological treatment options for NAFLD/NASH. However, as the pathogenesis of NAFLD is further unravelled, targeted treatment options are being investigated [5]. Among these, peroxisome proliferator-activated receptor (PPAR) agonists may be clinically relevant in this respect by reducing insulin resistance and stimulating fat redistribution, including from visceral to peripheral adipose tissue and from liver and muscle to adipose tissue [8]. Agonists of PPAR- γ decrease hyperglycaemia through improved fatty acid uptake by adipose tissue and enhanced β -cell function and insulin sensitivity [8]. Both PPAR- α and PPAR- γ are expressed on vascular endothelial cells, PPAR- α is primarily expressed in the liver and PPAR- γ is mainly expressed in hepatic immune cells and in endothelial and smooth muscle cells [9]. Both receptors can mediate anti-inflammatory effects [8,9]. Several studies have proposed that PPAR agonists may improve clinical and histological features of NASH and NASH-fibrosis [10]. Most studies have involved the use of pioglitazone, a PPAR- γ agonist, showing beneficial effects on NASH in both diabetic and non-diabetic patients [10–13]. Recent data suggest beneficial effects of a dual PPAR- α/γ agonist (Saroglitazar) on NAFLD/NASH in two phase II trials with respectively 106 and 16 subjects, and a phase IIb trial with a PPAR pan agonist (Lanifibranor) in 247 subjects [14–16]. Another potent dual PPAR agonist with an affinity for both PPAR- α and PPAR- γ subtypes is aleglitazar [17]. This drug was previously investigated in the AleCardio trial (effect of aleglitazar on Cardiovascular outcomes after acute coronary syndrome in patients with type 2 diabetes mellitus), which compared the effects of aleglitazar with placebo on cardiovascular morbidity and mortality among patients with type 2 diabetes (T2DM) and acute coronary syndrome (NCT01042769, www.clinicaltrials.gov) [18]. The trial was terminated prematurely due to lack of efficacy to reduce cardiovascular risks and safety concerns [18]. Several other PPAR agonists

are still being developed (Saroglitazar, Lanifibranor) for the treatment of NAFLD. Therefore, we believe the data of the Alecardio trial to be of importance as it gives the opportunity to evaluate possible beneficial effects of PPAR- α/γ agonists on NAFLD and associated fibrosis in a large number of patients. For this purpose, non-invasive tests (NITs) as indirect indicators of liver fibrosis and steatosis, were studied at several time points of the trial. This analysis serves as a proof of concept of PPAR- α/γ agonists pathway as target for NAFLD/NASH treatment.

Methods

Study design and study population

This is a post-hoc analysis of the AleCardio Trial, a large randomized, double-blind, placebo-controlled, multi-centre trial [18]. The detailed design and primary outcomes have been published previously [17]. In brief, eligible patients were randomized to receive aleglitazar (150 mg daily), or matching placebo added to standard medical care. Patients were considered eligible if they were hospitalized for acute coronary syndrome with either previously or newly diagnosed T2DM. Simultaneous use of systemic corticosteroids for longer than 2 weeks, thiazolidinediones, or fibrates was not allowed. Patients returned for outpatient visits at 1,3,6,9,12, 18 and 24 months following randomization. Information on the randomization process and exclusion criteria has been extensively described in the published design and outcome reports [17,18]. After a median follow-up of 2 years, the trial was ended prematurely due to futility for efficacy and in response to a higher incidence of safety concerns in the aleglitazar group, including heart failure, gastro-intestinal hemorrhages and renal dysfunction.

Outcomes and definitions

The primary outcome of this post-hoc analysis was the change in biochemical indicators (proxies) for liver steatosis and fibrosis over time as measured by algorithms based on biometric data and serum markers. Several serum markers have shown an adequate diagnostic accuracy for liver steatosis and fibrosis. Based on the guidelines of the European Association for the Study of the Liver, two scores reflecting risk of having steatosis were chosen: the liver fat score (LFS) and the liver accumulation product (LAP; S1 Table) [1,19,20]. The cut-off value for LFS is -0.640 and for $\ln\text{LAP} >4$ for males and >4.4 for women. To assess liver fibrosis, the NAFLD fibrosis score (NFS), and the fibrosis 4 score (FIB-4) were used (S1 Table). The NFS is divided into three categories; low ($\text{NFS} < -1.455$), indeterminate ($\text{NFS} \geq -1.455$ and $\text{NFS} \leq 0.675$) and high ($\text{NFS} > 0.675$). The FIB-4 score is divided into 3 categories; low ($\text{FIB-4} \leq 1.30$), middle ($\text{FIB-4} > 1.30$ and < 2.67), and high ($\text{FIB-4} \geq 2.67$). These scores have been extensively validated in a broad spectrum of NAFLD patients [1]. Secondary outcomes included the ratio of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and body mass index (BMI) [21].

Statistical methods

All statistical analyses were performed using SAS statistical software version 9.4 (SAS Institute, Cary, NC, USA). We tabulated baseline characteristics and markers of liver fibrosis risk stratified by treatment arm.

The distributions of liver fibrosis risk, AST, ALT and BMI were checked. For normally distributed variables mean \pm SD is reported while for not normally distributed variables the median with IQR is given. We examined absolute levels and changes in liver fibrosis risk markers compared to baseline until 24 months and compared these between the treatments arms using ANOVA test or Kruskal-Wallis test for continuous variables. Furthermore, we calculated

the proportion of participants in the pre-specified NFS and FIB-4 categories and examined the proportion that shifted from a higher category at baseline to a lower category during the study and vice versa per treatment group. Differences in proportions between treatment groups were compared using Chi-square for categorical variables. We calculated the distribution free 95% CI for variables that we reported the median with IQR for (i.e. LFS), as it may be inappropriate to calculate distribution-dependent confidence intervals when the assumed distribution does not fit the data well.

Results

Patients

Patients were included between February 2010 and May 2012 from 720 sites in 26 countries. Of the 7,226 patients who underwent randomization, 3,616 were assigned to receive aleglitazar and 3,610 were assigned to receive placebo. Baseline characteristics including NITs at baseline are described in Table 1. The majority of patients had overweight (median BMI 28.6 kg/m² in the treatment group and 28.7 kg/m² in placebo group). The mean HbA1c was 7.8% (62 mmol/mol) for both groups. Oral glucose lowering drugs were used in 79% of aleglitazar and 78% in the placebo group, and insulin was used in 29% and 30%, respectively. In the treatment group 20% of patients were classified at baseline as having a high risk of fibrosis based on the NFS and 19% in the placebo group. The median follow up period was 104 weeks (interquartile range, IQR: 82–129 weeks). More patients in the treatment group discontinued the study drug prematurely compared to the placebo group (29.3% for aleglitazar versus 25.3% for placebo).

Outcomes

The change in NITs for liver steatosis and fibrosis in the treatment and placebo groups are shown in Fig 1. NITs for both steatosis (LFS, LAP) and fibrosis (FIB-4) in the treatment group showed an initial steep decline between 3 and 6 months, followed by a slow gradual increase up to the end of the study period (24 months), whereas scores in the placebo group remained unchanged or increased. Change from baseline for LFS, LAP and FIB-4 remained significantly lower in the treatment group compared to placebo ($P < 0.001$ for all proxies at all time points ≥ 3 months). The median LFS was decreased by -0.20 (95% CI -0.26, -0.16) in the treatment group and increased by 0.17 (95% CI 0.12–0.22) in the placebo group after 24 months. Mean LAP was decreased by -5.12 (95% CI -7.3, -3.0) after 24 months of treatment and was increased by 14.4 (95% CI 11.5, 17.3) after 24 months of placebo use. After 24 months, the mean change in FIB-4 was -0.26 (95% CI -0.22, -0.30) in the treatment group vs. 0.05 (95% CI 0.01, 0.09) for placebo (Table 2). The mean change in NFS in the treatment group was 0.13 (95% CI 0.09, 0.17) vs. 0.33 (95% CI 0.27, 0.39) for placebo. NFS was decreased in the treatment group up until 12 months compared to placebo ($P < 0.001$), however at 24 months in both the treatment and placebo group NFS was higher than baseline measurement (Table 2). The ALT/AST ratio, also decreased more strongly in the treatment group (-0.31 [95% CI -0.33, -0.29]) compared to the placebo group (-0.03 [95% CI -0.09, -0.05]) from baseline to month 3 ($P < 0.001$). (Fig 2). This decrease at 3 months continued throughout the subsequent visits. BMI increased in both intervention groups from baseline to 24 months, but this increase was larger in the treatment group than in the placebo group (1.73 [95% CI 1.63, 1.83] kg/m² vs. 0.41 [95% CI 0.33, 0.49] kg/m² respectively, $P < 0.001$). Overall, there were no differences in response to therapy per region (data not shown).

Fig 3 shows the change in FIB-4 and NFS categories from baseline to month 24. In the treatment group more participants showed improvement by shifting to a lower FIB-4 and NFS category compared to placebo (FIB-4: 25% vs. 14%, $P < 0.001$ and NFS: 11% vs. 9%, $P < 0.004$, for

Table 1. Baseline characteristics of the patients in the aleglitazar group compared with the placebo group.

Characteristics	N (%)	
	Aleglitazar group (n = 3616)	Placebo group (n = 3610)
Age, years [mean \pm SD]	61 \pm 10	61 \pm 10
Men [n (%)]	2,641 (73)	2,619 (73)
Race/ethnicity		
White [n (%)]	2,427 (67)	2,391 (66)
Asian [n (%)]	942 (26)	942 (26)
Smoking status		
Never smoked [n (%)]	1,422 (39)	1,363 (38)
Past/Current [n (%)]	2,189 (61)	2,241 (62)
Clinical values		
Body weight, kg [median (IQR)]	80.6 (70.0–93.6)	81.0 (70.0–94.1)
BMI, kg/m ² [median, (IQR)]	28.6 (25.6–32.1)	28.7 (25.7–32.5)
Waist circumference, cm [mean \pm SD]	102 \pm 14	102 \pm 14
Waist/hip ratio [mean \pm SD]	0.97 \pm 0.08	0.97 \pm 0.08
HbA1c [mean \pm SD]	7.8 \pm 1.7	7.8 \pm 1.6
HOMA-IR [median (IQR)]	3.4 (2.0–6.0)	3.3 (2.0–6.2)
Fasting plasma glucose, mmol/L [median (IQR)]	7.5 (6.3–9.5)	7.4 (6.2–9.5)
Insulin, μ U/L [median (IQR)]	9.8 (6.1–16.3)	9.6 (6.1–16.2)
Triglycerides, mmol/L [mean \pm SD]	1.72 \pm 1.02	1.74 \pm 1.14
HDL cholesterol, mmol/L [mean \pm SD]	1.09 \pm 0.27	1.08 \pm 0.29
LDL cholesterol, mmol/L [mean \pm SD]	2.04 \pm 0.80	2.06 \pm 0.80
Aspartate Transaminase (AST), U/L [mean \pm SD]	28 \pm 19	29 \pm 21
Alanine Transaminase (ALT), U/L [mean \pm SD]	23 \pm 13	23 \pm 14
ASAT/ALAT ratio [mean \pm SD]	1.22 \pm 0.41	1.21 \pm 0.42
Platelets, $\times 10^9/L$ [mean \pm SD]	264 \pm 84	272 \pm 93
Albumin, g/dL [mean \pm SD]	4.0 \pm 0.4	4.0 \pm 0.4
eGFR, mL/min/1.73m ² [mean \pm SD]	77 \pm 20	78 \pm 20
SBP, mmHg [mean \pm SD]	128 \pm 17	128 \pm 18
DBP, mmHg [mean \pm SD]	76 \pm 10	76 \pm 10
Medical history		
Duration of diabetes, years [median (IQR)]	6.5 (2.8–11.9)	6.5 (2.7–11.8)
CHD (MI, revascularization or angina) [n, %]	1,460 (40)	1,475 (41)
Stroke or TIA [n, %]	265 (7.3)	296 (8.2)
Retinopathy [n, %]	190 (5.3)	189 (5.2)
Medication use		
Sulphonylurea [n, %]	1,250 (35)	1,222 (34)
Metformin [n, %]	2,414 (67)	2,379 (66)
Insulin [n, %]	1,035 (29)	1,066 (30)
Antiplatelet medication [n, %]	3,108 (86)	3,106 (86)
ACE inhibitors or ARB [n, %]	2,936 (81)	2,923 (81)
Statins [n, %]	3,332 (92)	3,357 (93)
Medication affecting renal function [n, %]	3,100 (86)	3,085 (85)
Diuretics [n, %]	1,134 (31)	1,142 (32)
NAFLD Markers		
FIB4 score		
1.30–2.67 [n, %]	1,342 (41)	1,254 (38)

(Continued)

Table 1. (Continued)

Characteristics	N (%)	
	Aleglitazar group (n = 3616)	Placebo group (n = 3610)
= <1.30 [n, %]	1,695 (52)	1,797 (55)
> = 2.67 [n, %]	213 (7)	210 (6)
NAFLD Fibrosis Score (NFS)		
Low (< -1.455) [n, %]	588 (18)	680 (21)
Indeterminate (-1.455–0.675) [n, %]	1,998 (62)	1,930 (60)
High (> 0.675) [n, %]	648 (20)	630 (19)
Liver Fat Score (LFS) [median (IQR)]	0.63 (-0.05–1.78)	0.60 (-0.03–1.73)
Liver Accumulation Product (LAP) [mean \pm SD]	68 \pm 50	69 \pm 64
lnLAP (natural logarithm) [mean \pm SD]	4.2 \pm 3.9	4.2 \pm 4.2

Note: Baseline characteristics, except for NITs, have been reported previously [17].

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aleglitazar vs. placebo) The opposite for worsening was also shown; less participants in the treatment group showed worsening by shifting to a higher FIB-4 and NFS category than in the placebo group (FIB-4: 8% vs. 18%, $P < 0.001$ and NFS: 15% and 20%, $P = 0.024$, for aloglitazar vs. placebo). Fig 4 shows the change in LAP and LFS with respect to their cut-off values from baseline to month 24. For LAP more participants in the treatment group showed improvement with respect to the cut-off value as compared to the placebo group (17% vs. 9%, $P < 0.001$, for aloglitazar vs. placebo). Again, for LAP also less worsening was seen in the treatment group in respect to the cut-off value (9% vs. 15%, $P < 0.001$, for aloglitazar vs. placebo). For LFS slightly less worsening was shown (2.5% vs. 3.9%, $P < 0.031$, for aloglitazar vs. placebo), however no

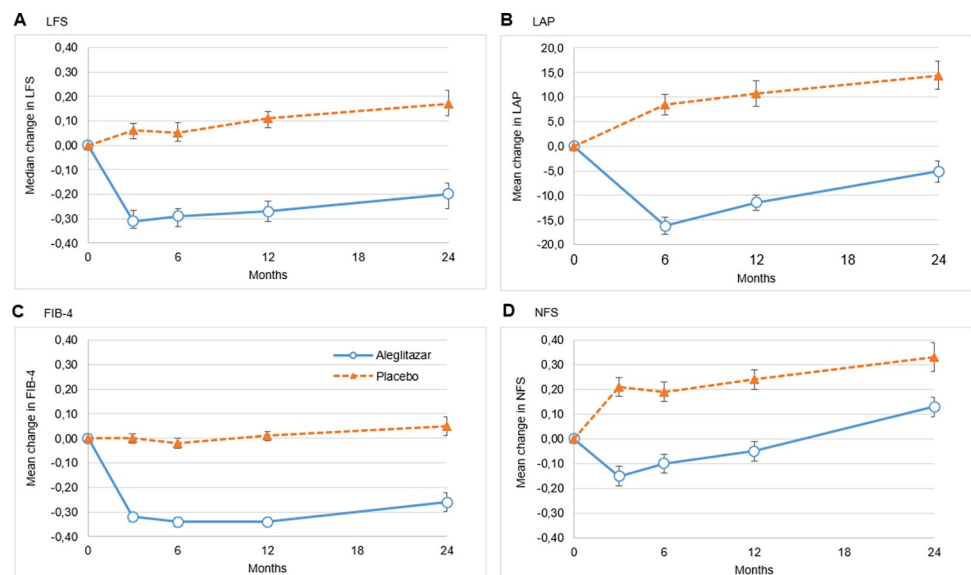


Fig 1. Change in NITs of liver steatosis and fibrosis from baseline. Value at baseline for A: Median LFS of 0.60 for placebo and 0.63 for aloglitazar, B: Mean LAP of 69.5 for placebo and 67.8 for aloglitazar, C: Mean FIB-4 of 1.40 for placebo and 1.44 for aloglitazar, D: Mean NFS of -0.50 for placebo and -0.41 for aloglitazar. Error bars indicate 95% CIs. Change from baseline is significantly different in the aloglitazar and the placebo group at all timepoints ≥ 3 months for all proxies (all $P < 0.001$).

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Table 2. Changes in absolute values of non-invasive tests over time.

Visits (months)	LFS ^{†‡}		LAP ^{†‡}		NFS ^{†‡}		FIB-4 ^{†‡}	
	Aleglitazar	Placebo	Aleglitazar	Placebo	Aleglitazar	Placebo	Aleglitazar	Placebo
0	N = 3,057 0.63 (0.57, 0.69)	N = 3,015 0.60 (0.56, 0.66)	N = 3,496 67.8 (66.2, 69.5)	N = 3,478 69.5 (67.3, 71.6)	N = 3,234 -0.41 (-0.45, -0.37)	N = 3,240 -0.50 (-0.56, -0.40)	N = 3,250 1.44 (1.42, 1.46)	N = 3,261 1.40 (1.38, 1.42)
3	N = 2,877 0.17 (0.13, 0.20)	N = 2,855 0.63 (0.57, 0.70)	Not available	Not available	N = 3,057 -0.59 (-0.63, -0.55)	N = 3,020 -0.29 (-0.33, -0.25)	N = 3,080 1.11 (1.09, 1.13)	N = 3,047 1.41 (1.39, 1.43)
6	N = 2,743 0.19 (0.16, 0.23)	N = 2,761 0.66 (0.59, 0.72)	N = 3,066 51.0 (49.2, 52.8)	N = 3,096 78.3 (75.5, 81.0)	N = 2,909 -0.54 (-0.58, -0.50)	N = 2,943 -0.31 (-0.35, -0.27)	N = 2,931 1.10 (1.08, 1.12)	N = 2,964 1.39 (1.37, 1.41)
12	N = 2,620 0.20 (0.15, 0.23)	N = 2,674 0.68 (0.62, 0.74)	N = 2,932 55.4 (53.8, 57.0)	N = 2,994 80.4 (77.8, 83.0)	N = 2,765 -0.50 (-0.54, -0.46)	N = 2,818 -0.25 (-0.29, -0.21)	N = 2,786 1.10 (1.08, 1.12)	N = 2,840 1.44 (1.42, 1.46)
24	N = 1,634 0.22 (0.17, 0.28)	N = 1,707 0.67 (0.61, 0.75)	N = 1,725 60.7 (58.3, 63.1)	N = 1,783 83.8 (80.3, 87.4)	N = 1,720 -0.37 (-0.43, -0.31)	N = 1,799 -0.20 (-0.26, -0.14)	N = 1,731 1.16 (1.14, 1.18)	N = 1,817 1.47 (1.43, 1.51)

†LFS = liver fat score; LAP = liver accumulation product; NFS = Non-alcoholic fatty liver disease fibrosis score; FIB-4 = fibrosis 4 calculator.

‡ Values of LFS are presented as median (distribution free 95% CI) and values of LAP, NFS and FIB-4 as mean (95% CI).

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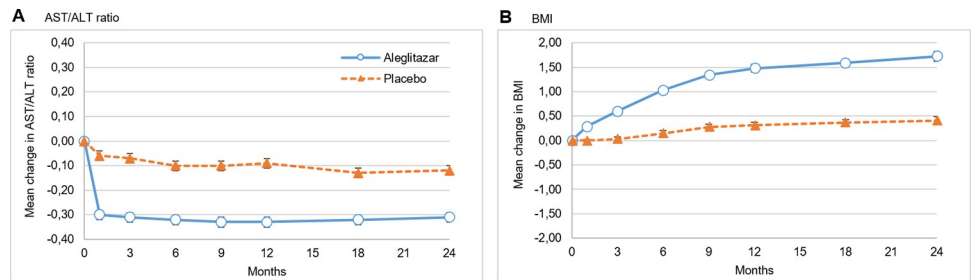


Fig 2. Change in AST/ALT ratio and BMI from baseline. Value at baseline for **A**: Mean AST/ALT of 1.21 for placebo and 1.22 for aleglitazar, **B**: Mean BMI of 29.5 kg/m² for placebo and 29.3 kg/m² for aleglitazar. Error bars indicate 95% CIs. Change from baseline is significantly different in the aleglitazar and the placebo group at all timepoints ≥ 1 month for both AST/ALT ratio and BMI (all $P < 0.001$).

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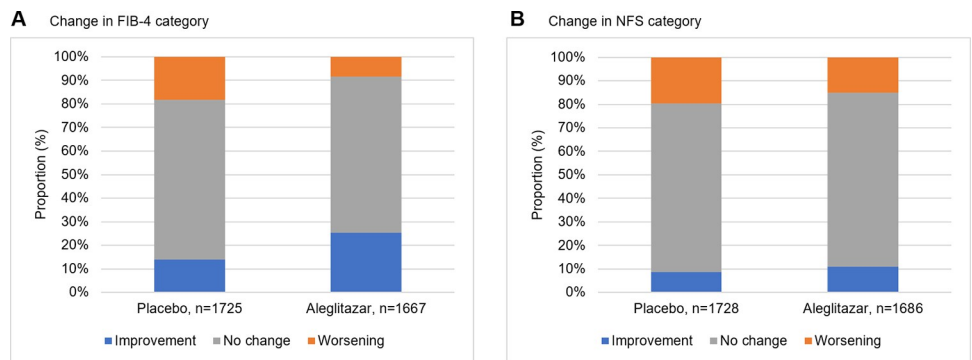


Fig 3. Change in FIB-4 and NFS category from baseline until 24 months follow-up. Proportion of randomized patients that either improved (changed to a lower category), worsened (changed to a higher category) or did not change in category. Proportion of change is significantly different between treatment and placebo for FIB-4 (**A**) and NFS (**B**). FIB-4: Both improvement and worsening $P < 0.001$, NFS: Improvement $P < 0.004$, worsening $P = 0.024$.

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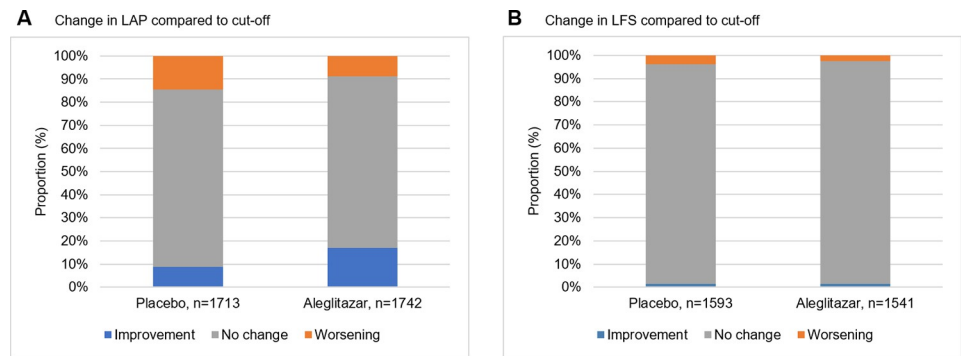


Fig 4. Change in LFS and LAP compared to cut-off values at baseline until 24 months follow-up. Proportion of randomized patients that either improved (lnLAP below cut-off <4 for males and <4.4 for females, LFS below cut-off <-0.640), worsened (lnLAP above cut-off >4 for males and >4.4 for females, LFS above cut-off >-0.064), or did not change in respect to respective cut-off value. Proportion of change is significantly different between treatment and placebo for LAP (A), both improvement and worsening $P < 0.001$. For LFS (B) only worsening is significantly less in the treatment group, improvement $P = 0.974$, worsening $P = 0.031$.

<https://doi.org/10.1371/journal.pone.0277706.g004>

change was observed on improvement in respect to the cut-off (1.5% vs. 1.5%, $P = 0.0974$, aleglitazar vs. placebo).

Discussion

The results of this post-hoc analysis provide evidence that a dual PPAR agonist with an affinity for the PPAR- α and PPAR- γ may have a beneficial effects on NAFLD and subsequent risk of fibrosis in patients with T2DM. Our results show an early improvement of NITs for liver fibrosis and steatosis upon initiation of aleglitazar compared to placebo, and persistence of this improvement. This effect lasted throughout the treatment period (except for NFS). In both, the treatment group as well as the placebo group there was a gradual increase of NITs during follow-up. This trend may be due to the progression of insulin resistance in these patients. Noticeably, BMI increased in those randomized to aleglitazar, which is likely due to redistribution of body fluids and subcutaneous fat [22,23]. We hypothesize that the persistence of improvement at 24 months of treatment which was not seen in the NFS score is due to increased BMI, since this variable is included in the NFS algorithm. Our analysis in a large number of subjects, expands the recently published results with a similar compound, saroglitazar, in 106 and 16 subjects with NAFLD/NASH [15].

A recent meta-analysis reported that next to bariatric surgery, treatment with a single PPAR- γ agonist (pioglitazone) was most effective in patients with NASH for steatosis and reduction of lobular inflammation [24]. Previous literature has mainly focused on these PPAR- γ agonists, with rosiglitazone in addition to pioglitazone as most frequently reported. These studies showed favourable effects on NASH and even on liver fibrosis [11,12,24]. One of the largest randomized trials involving 247 patients with NASH, the PIVENS trial, compared the use of pioglitazone, vitamin E and placebo with histological improvement of NASH as their outcome [13]. This study reported a higher rate of improvement of liver histology compared to placebo (34% vs. 19%), although this difference did not reach statistical significance; effects of vitamin E did reach significance with an improvement rate of 43%. Remarkably, after discontinuation of both drugs, liver enzymes increased again. While this is an interesting and relevant finding, data after discontinuation of aleglitazar are not available in our cohort. An important difference with PIVENS compared to AleCardio is that none of the patients in PIVENS had T2DM, a highly relevant comorbid condition of NAFLD. Another randomized,

placebo-controlled trial included patients with T2DM, in addition to subjects with prediabetes and showed that 51% of participants using pioglitazone had resolution of NASH compared to 19% in the placebo group [12]. It should be noted that all patients were additionally prescribed a hypocaloric diet. The combination of therapies, such as vitamin E or a hypocaloric diet might be of added benefit in the treatment of NASH, as its complex pathogenesis could render monotherapy insufficiently effective [6,11]. In line with previously published studies on PPAR- γ agonists and the recent papers with saroglitazar and lanifibranor, we found an increase in BMI in the treatment group. This increase in BMI may have been partly caused by fluid retention, as is frequently seen with these compounds, with the exception of saroglitazar as reported by Gawrieh et al. [14].

While overall NITs decreased in our cohort, the average absolute decrease was small in this population. This could be due to the relatively low percentage of patients with advanced stages of NAFLD at baseline reflected by FIB-4 > 2.67. Results of a study on the safety and efficacy of elafibranor, a dual PPAR- α/δ agonist, are in line with our findings showing a lower efficacy in mild NAFLD [25]. It has been proposed that PPAR- α agonists have a higher efficacy in more severe NAFLD, as hepatic PPAR- α expression is lower in advanced NASH and liver fibrosis [26]. Treatment with lanifibranor, a pan PPAR agonist in patients with severe active NASH led to lowering of a histologically assessed score of NASH activity, also in a subset of diabetic patients [16].

In an interim analysis of the original trial, aleglitazar did not improve the primary cardiovascular efficacy endpoint, while occurrence of safety endpoints, especially renal dysfunction, was higher in the treatment group. Two trials on elafibranor, a PPAR- α/δ agonist, have been terminated prematurely due to lack of efficacy for primary endpoints as well, although safety issues did not seem to play a role in the decision there [27,28]. Two other compounds (Saroglitazar and Lanifibranor) are still being developed for the treatment of NAFLD/NASH and no safety concerns have been reported so far. Saroglitazar has been approved in India since 2013 for treatment of diabetic dyslipidemia and in post-marketing real-world evidence studies in this population saroglitazar has shown to improve liver parameters as well [29,30].

With data on a total of 7,226 patients, this is the largest cohort reporting on the effect of a dual PPAR agonist on NAFLD in a randomized comparison. Since patients were included from several centers in different countries, it represents a broad spectrum of patients at risk of NAFLD. However, some limitations need to be acknowledged. This is a post-hoc analysis, in which NITs of liver steatosis and fibrosis were not planned outcomes of this study. Moreover, no histological liver samples were obtained. However, NFS and FIB-4 have been extensively validated and both have an acceptable diagnostic accuracy as measured by an area under the curve of 0.84 and 0.81 respectively. Both, FIB-4 and NFS were shown to increase in a stepwise manner corresponding to each fibrosis stage in a large cohort of Caucasian biopsy-proven NAFLD patients [31]. There is also an increasing body of evidence demonstrating the effect of these NITs in clinical trials. For example in the TONIC study, each unit decrease in ALT was associated with a 30% increased odds of histological improvement [32]. In the FLINT trial, patients with histologic fibrosis improvement at week 24 demonstrated reductions in APRI, FIB4, and NFS [33]. The European Association for the Study of Liver diseases (EASL) recommends using NITs to screen patients at risk and for risk stratification [1,34], however, the use of NITs to monitor fibrosis progression/regression in clinical trials (in combination with other non-invasive methods like imaging) still requires validation. Following the EASL guideline, more than half of the participants were eligible for further evaluation based on a FIB-4 cut-off value of 1.30. As previously noted, the levels of the NITs for liver fibrosis were relatively low in our cohort. In contrast, the NITs for liver steatosis were relatively higher in this cohort. At baseline the cohort showed values at around the LAP cut-off of <4 for males and <4.4 for

females (average InLAP of 4.2 in cohort) where scores above cut-off indicate moderate or severe steatosis and below cut-off no indication of steatosis [19]. LFS seems to have low discriminative power in our study, almost the complete cohort (96%) was above cut-off value of <-0.0640 at baseline, which indicates increased liver fat and thus NAFLD [20]. As all patients were obese and had T2DM, we consider this cohort to be a representative spectrum of patients at high risk of NAFLD. Our study indicates that a different cut-off value for LFS may be more informative in a high-risk population. Lastly, alcohol intake was not quantitated in detail, however excessive alcohol use is not considered to be an issue in our analysis since alcohol dependency was an exclusion criterion of the trial.

In conclusion, this large randomized comparison shows a potential favourable effect of a dual PPAR- α/γ agonist on NITs for liver steatosis and fibrosis in a high-risk population for NAFLD of overweight patients with T2DM. Future studies investigating PPAR agonists should focus on patients with severe disease as well as the possibility of combination of therapies. These results warrant further investigation of the efficacy of dual PPAR agonists as treatment for patients with NAFLD.

Supporting information

S1 Table. Scores predicting NAFLD severity.
(DOCX)

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References

1. EASL, EASD, EASO. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol.* 2016; 64(6):1388–402. <https://doi.org/10.1016/j.jhep.2015.11.004> PMID: 27062661.
2. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology.* 1999; 116(6):1413–9. [https://doi.org/10.1016/s0016-5085\(99\)70506-8](https://doi.org/10.1016/s0016-5085(99)70506-8) PMID: 10348825.
3. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology.* 2017; 65(5):1557–65. <https://doi.org/10.1002/hep.29085> PMID: 28130788; PubMed Central PMCID: PMC5397356.

4. Duell PB, Welty F. K., Miller M., Chait A., Hammond G., Ahmad Z, et al. Nonalcoholic Fatty Liver Disease and Cardiovascular Risk: A Scientific Statement From the American Heart Association. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2022; 42(6):168–e85. <https://doi.org/10.1161/ATV.000000000000153> PMID: 35418240
5. Ganguli S, DeLeeuw P, Satapathy SK. A Review Of Current And Upcoming Treatment Modalities In Non-Alcoholic Fatty Liver Disease And Non-Alcoholic Steatohepatitis. *Hepat Med*. 2019; 11:159–78. <https://doi.org/10.2147/HMER.S188991> PMID: 31814783; PubMed Central PMCID: PMC6863115.
6. Dufour JF, Caussy C, Loomba R. Combination therapy for non-alcoholic steatohepatitis: rationale, opportunities and challenges. *Gut*. 2020; 69(10):1877–84. <https://doi.org/10.1136/gutjnl-2019-319104> PMID: 32381514; PubMed Central PMCID: PMC7497577.
7. Chavez-Tapia NC, Tellez-Avila FI, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Cervera J, Uribe M. Bariatric surgery for non-alcoholic steatohepatitis in obese patients. *Cochrane Database Syst Rev*. 2010;(1):CD007340. <https://doi.org/10.1002/14651858.CD007340.pub2> PMID: 20091629; PubMed Central PMCID: PMC7208314.
8. Skat-Rordam J, Hojland Ipsen D, Lykkesfeldt J, Tveden-Nyborg P. A role of peroxisome proliferator-activated receptor gamma in non-alcoholic fatty liver disease. *Basic Clin Pharmacol Toxicol*. 2019; 124(5):528–37. <https://doi.org/10.1111/bcpt.13190> PMID: 30561132; PubMed Central PMCID: PMC6850367.
9. Tsai HC, Li TH, Huang CC, Huang SF, Liu RS, Yang YY, et al. Beneficial Effects of the Peroxisome Proliferator-Activated Receptor alpha/gamma Agonist Alogliptazar on Progressive Hepatic and Splanchnic Abnormalities in Cirrhotic Rats with Portal Hypertension. *Am J Pathol*. 2018; 188(7):1608–24. <https://doi.org/10.1016/j.ajpath.2018.03.018> PMID: 29929914.
10. Musso G, Cassader M, Paschetta E, Gambino R. Thiazolidinediones and Advanced Liver Fibrosis in Nonalcoholic Steatohepatitis: A Meta-analysis. *JAMA Intern Med*. 2017; 177(5):633–40. <https://doi.org/10.1001/jamainternmed.2016.9607> PMID: 28241279; PubMed Central PMCID: PMC5470366.
11. Bril F, Kalavalapalli S, Clark VC, Lomonaco R, Soldevila-Pico C, Liu IC, et al. Response to Pioglitazone in Patients With Nonalcoholic Steatohepatitis With vs Without Type 2 Diabetes. *Clin Gastroenterol Hepatol*. 2018; 16(4):558–66 e2. <https://doi.org/10.1016/j.cgh.2017.12.001> PMID: 29223443.
12. Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, et al. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. *Ann Intern Med*. 2016; 165(5):305–15. <https://doi.org/10.7326/M15-1774> PMID: 27322798.
13. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med*. 2010; 362(18):1675–85. <https://doi.org/10.1056/NEJMoa0907929> PMID: 20427778; PubMed Central PMCID: PMC2928471.
14. Gawrieh S, Noureddin M, Loo N, Mohseni R, Awasty V, Cusi K, et al. Saroglitazar, a PPAR-alpha/gamma Agonist, for Treatment of NAFLD: A Randomized Controlled Double-Blind Phase 2 Trial. *Hepatology*. 2021; 74(4):1809–24. Epub 2021/04/04. <https://doi.org/10.1002/hep.31843> PMID: 33811367.
15. Siddiqui MS, Idowu MO, Parmar D, Borg BB, Denham D, Loo NM, et al. A Phase 2 Double Blinded, Randomized Controlled Trial of Saroglitazar in Patients With Nonalcoholic Steatohepatitis. *Clin Gastroenterol Hepatol*. 2021; 19(12):2670–2. Epub 2020/11/06. <https://doi.org/10.1016/j.cgh.2020.10.051> PMID: 33152542.
16. Francque SM, Bedossa P, Ratziu V, Anstee QM, Bugianesi E, Sanyal AJ, et al. A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. *N Engl J Med*. 2021; 385(17):1547–58. Epub 2021/10/21. <https://doi.org/10.1056/NEJMoa2036205> PMID: 34670042.
17. Lincoff AM, Tardif JC, Neal B, Nicholls SJ, Ryden L, Schwartz GG, et al. Evaluation of the dual peroxisome proliferator-activated receptor alpha/gamma agonist alogliptazar to reduce cardiovascular events in patients with acute coronary syndrome and type 2 diabetes mellitus: rationale and design of the AleCardio trial. *Am Heart J*. 2013; 166(3):429–34. <https://doi.org/10.1016/j.ahj.2013.05.013> PMID: 24016490.
18. Lincoff AM, Tardif JC, Schwartz GG, Nicholls SJ, Ryden L, Neal B, et al. Effect of alogliptazar on cardiovascular outcomes after acute coronary syndrome in patients with type 2 diabetes mellitus: the AleCardio randomized clinical trial. *JAMA*. 2014; 311(15):1515–25. <https://doi.org/10.1001/jama.2014.3321> PMID: 24682069.
19. Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. *BMC Gastroenterol*. 2010; 10:98. <https://doi.org/10.1186/1471-230X-10-98> PMID: 20738844; PubMed Central PMCID: PMC2940930.
20. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology*. 2009; 137(3):865–72. <https://doi.org/10.1053/j.gastro.2009.06.005> PMID: 19524579.

21. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut*. 2010; 59(9):1265–9. <https://doi.org/10.1136/gut.2010.216077> PMID: 20801772.
22. Thiazolidinediones Yki-Jarvinen H. *N Engl J Med*. 2004; 351(11):1106–18. Epub 2004/09/10. <https://doi.org/10.1056/NEJMr041001> PMID: 15356308.
23. Yang X, Smith U. Adipose tissue distribution and risk of metabolic disease: does thiazolidinedione-induced adipose tissue redistribution provide a clue to the answer? *Diabetologia*. 2007; 50(6):1127–39. Epub 2007/03/30. <https://doi.org/10.1007/s00125-007-0640-1> PMID: 17393135.
24. Panunzi S, Maltese S, Verrastro O, Labbate L, De Gaetano A, Pompili M, et al. Pioglitazone and bariatric surgery are the most effective treatments for non-alcoholic steatohepatitis: A hierarchical network meta-analysis. *Diabetes Obes Metab*. 2020. <https://doi.org/10.1111/dom.14304> PMID: 33368954.
25. Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, et al. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor- α and - δ , Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. *Gastroenterology*. 2016; 150(5):1147–59 e5. <https://doi.org/10.1053/j.gastro.2016.01.038> PMID: 26874076.
26. Francque S, Verrijken A, Caron S, Prawitt J, Paumelle R, Derudas B, et al. PPAR α gene expression correlates with severity and histological treatment response in patients with non-alcoholic steatohepatitis. *J Hepatol*. 2015; 63(1):164–73. <https://doi.org/10.1016/j.jhep.2015.02.019> PMID: 25703085.
27. Phase 3 Study to Evaluate the Efficacy and Safety of Elafibranor Versus Placebo in Patients With Non-alcoholic Steatohepatitis (NASH) (RESOLVE-IT) [Internet]. [cited Accessed May 18, 2022]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02704403?term=elafibranor&draw=2&rank=7>.
28. Study to Evaluate the Effect of Elafibranor on Hepatic Lipid Composition in Subjects With Nonalcoholic Fatty Liver (NAFL) [Internet]. [cited Accessed May 18, 2022]. Available from: <https://clinicaltrials.gov/ct2/show/record/NCT03953456?term=elafibranor&draw=2&rank=4>.
29. Goyal O, Nohria S, Goyal P, Kaur J, Sharma S, Sood A, et al. Saroglitazar in patients with non-alcoholic fatty liver disease and diabetic dyslipidemia: a prospective, observational, real world study. *Sci Rep*. 2020; 10(1):21117. Epub 2020/12/05. <https://doi.org/10.1038/s41598-020-78342-x> PMID: 33273703; PubMed Central PMCID: PMC7713236.
30. Rajesh NA, Drishya L, Ambati MMR, Narayanan AL, Alex M, R KK, et al. Safety and Efficacy of Saroglitazar in Nonalcoholic Fatty Liver Patients With Diabetic Dyslipidemia-A Prospective, Interventional, Pilot Study. *J Clin Exp Hepatol*. 2022; 12(1):61–7. Epub 2022/01/25. <https://doi.org/10.1016/j.jceh.2021.03.012> PMID: 35068786; PubMed Central PMCID: PMC8766544.
31. Younes R, Caviglia GP, Govaere O, Rosso C, Armandi A, Sanavia T, et al. Long-term outcomes and predictive ability of non-invasive scoring systems in patients with non-alcoholic fatty liver disease. *J Hepatol*. 2021; 75(4):786–94. Epub 2021/06/07. <https://doi.org/10.1016/j.jhep.2021.05.008> PMID: 34090928.
32. Vuppalanchi R, Jain AK, Deppe R, Yates K, Comerford M, Masuoka HC, et al. Relationship between changes in serum levels of keratin 18 and changes in liver histology in children and adults with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2014; 12(12):2121–30 e1-2. Epub 2014/05/23. <https://doi.org/10.1016/j.cgh.2014.05.010> PMID: 24846279; PubMed Central PMCID: PMC4830682.
33. Chalasani N, Abdelmalek MF, Loomba R, Kowdley KV, McCullough AJ, Dasarathy S, et al. Relationship between three commonly used non-invasive fibrosis biomarkers and improvement in fibrosis stage in patients with non-alcoholic steatohepatitis. *Liver Int*. 2019; 39(5):924–32. Epub 2018/09/27. <https://doi.org/10.1111/liv.13974> PMID: 30253043; PubMed Central PMCID: PMC6433535.
34. EASL. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis—2021 update. *J Hepatol*. 2021; 75(3):659–89. Epub 2021/06/25. <https://doi.org/10.1016/j.jhep.2021.05.025> PMID: 34166721.