

**PREVALENCE OF AND RISK FACTORS FOR FATTY  
LIVER IN THE GENERAL POPULATION:  
THE BAGNACAVALLO STUDY**

Giorgio Bedogni

[www.giorgiobedogni.it](http://www.giorgiobedogni.it)

## Note

- The present talk is based on version 0.0 of the MS

“PREVALENCE OF AND RISK FACTORS FOR FATTY LIVER IN THE GENERAL POPULATION OF NORTHERN ITALY: THE BAGNACAVALLO STUDY”

# Outline

- Background
- Aim
- Subjects and methods
- Results
- Discussion

# Outline

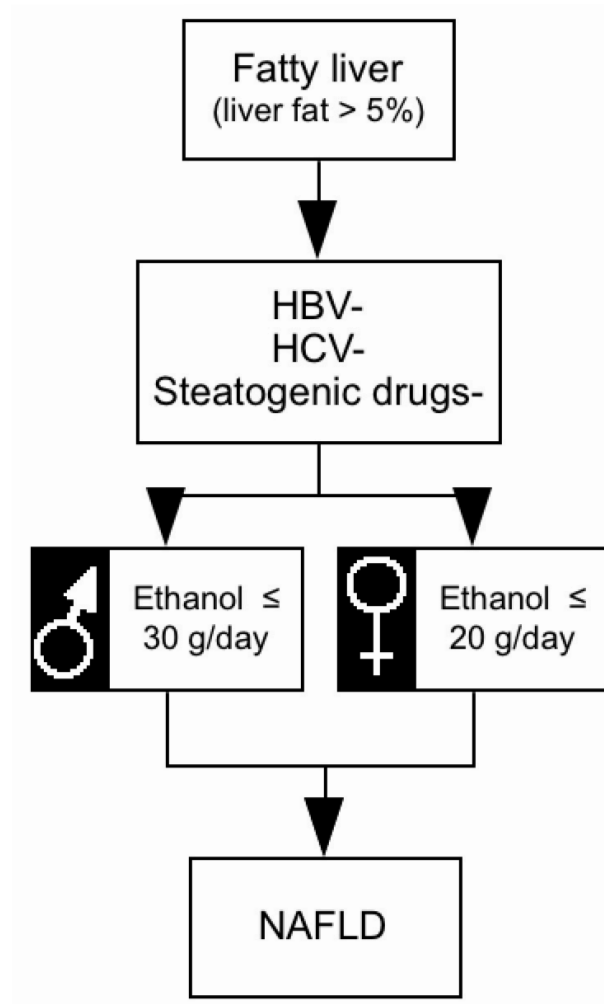
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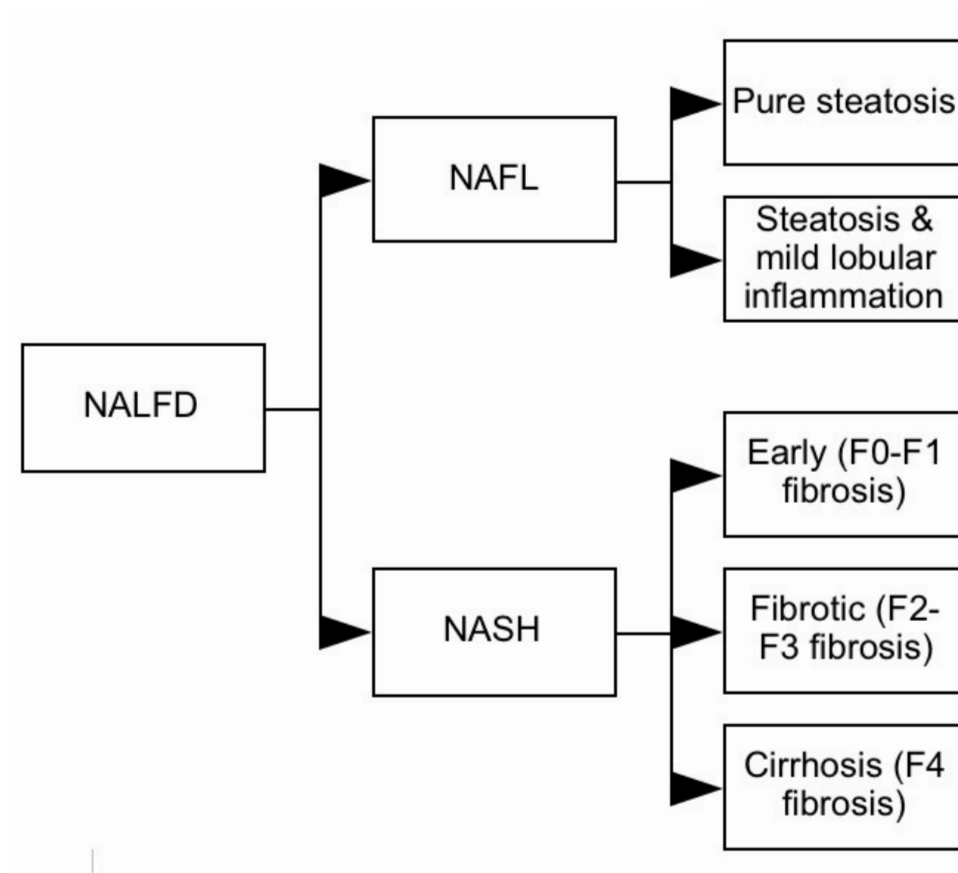
# Background

- Fatty liver (FL), the most common liver disease worldwide, is usually classified into non-alcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD) (1, 2).

# Background



# Background



# Background

	N	Studies	Fibrosis progression (years to progress 1 stage, mean [95%CI])
NAFLD	366	11	7.7 [5.5 to 14.8]
NAFL	133	6	14.3 [9.1 to 50.0]
NASH	116	7	7.1 [4.8 to 14.3]

Singh S Clin Gastroenterol Hepatol 2015;13:643  
(systematic review & metanalysis)

# Background

- The NAFLD *vs.* AFLD dichotomization is useful in clinical practice because ethanol is unlikely to be toxic at quantities  $\leq 30$  g/day but hides the important fact that ethanol and obesity do interact to determine the burden of liver disease in the general population (2–4).

# Background

- In the early 2000s, the Dionysos Study reported the first data on the prevalence and incidence of FL in the general population (5, 6).

# Prevalence of and Risk Factors for Nonalcoholic Fatty Liver Disease: The Dionysos Nutrition and Liver Study

Giorgio Bedogni,<sup>1,2</sup> Lucia Miglioli,<sup>2</sup> Flora Masutti,<sup>2</sup> Claudio Tiribelli,<sup>1,2</sup> Giulio Marchesini,<sup>3</sup> and Stefano Bellentani<sup>1,2,4</sup>

**The prevalence of and the risk factors for fatty liver have not undergone a formal evaluation in a representative sample of the general population.** We therefore performed a cross-sectional study in the town of Campogalliano (Modena, Italy), within the context of the Dionysos Project. Of 5,780 eligible persons aged 18 to 75 years, 3,345 (58%) agreed to participate in the study. Subjects with suspected liver disease (SLD), defined on the basis of elevated serum alanine aminotransferase (ALT) and  $\gamma$ -glutamyl-transferase (GGT) activity, hepatitis B surface antigen (HBsAg), or hepatitis C virus (HCV)-RNA positivity, were matched with randomly selected subjects of the same age and sex without SLD. A total of 311 subjects with and 287 without SLD underwent a detailed clinical, laboratory, and anthropometrical evaluation. Fatty liver was diagnosed by ultrasonography, and alcohol intake was assessed by using a 7-day diary. Multinomial logistic regression was used to detect risk factors for normal liver versus nonalcoholic fatty liver disease (NAFLD) and for alcoholic fatty liver (AFLD) versus NAFLD. **The prevalence of NAFLD was similar in subjects with and without SLD (25 vs. 20%,  $P = .203$ ).** At multivariable analysis, normal liver was more likely than NAFLD in older subjects and less likely in the presence of obesity, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and systolic hypertension; AFLD was more likely than NAFLD in older subjects, males, and in the presence of elevated GGT and hypertriglyceridemia, and less likely in the presence of obesity and hyperglycemia. **In conclusion, NAFLD is highly prevalent in the general population, is not associated with SLD, but is associated with many features of the metabolic syndrome.** (HEPATOLOGY 2005;42:44-52.)

# Background

- Many epidemiological studies on FL have been published since the Dionysos Study findings were made available (7).



# Background

- The worldwide prevalence of NAFLD was estimated to be 25% (95%CI 22 to 28%) by a recent meta-analysis of 45 studies (7).

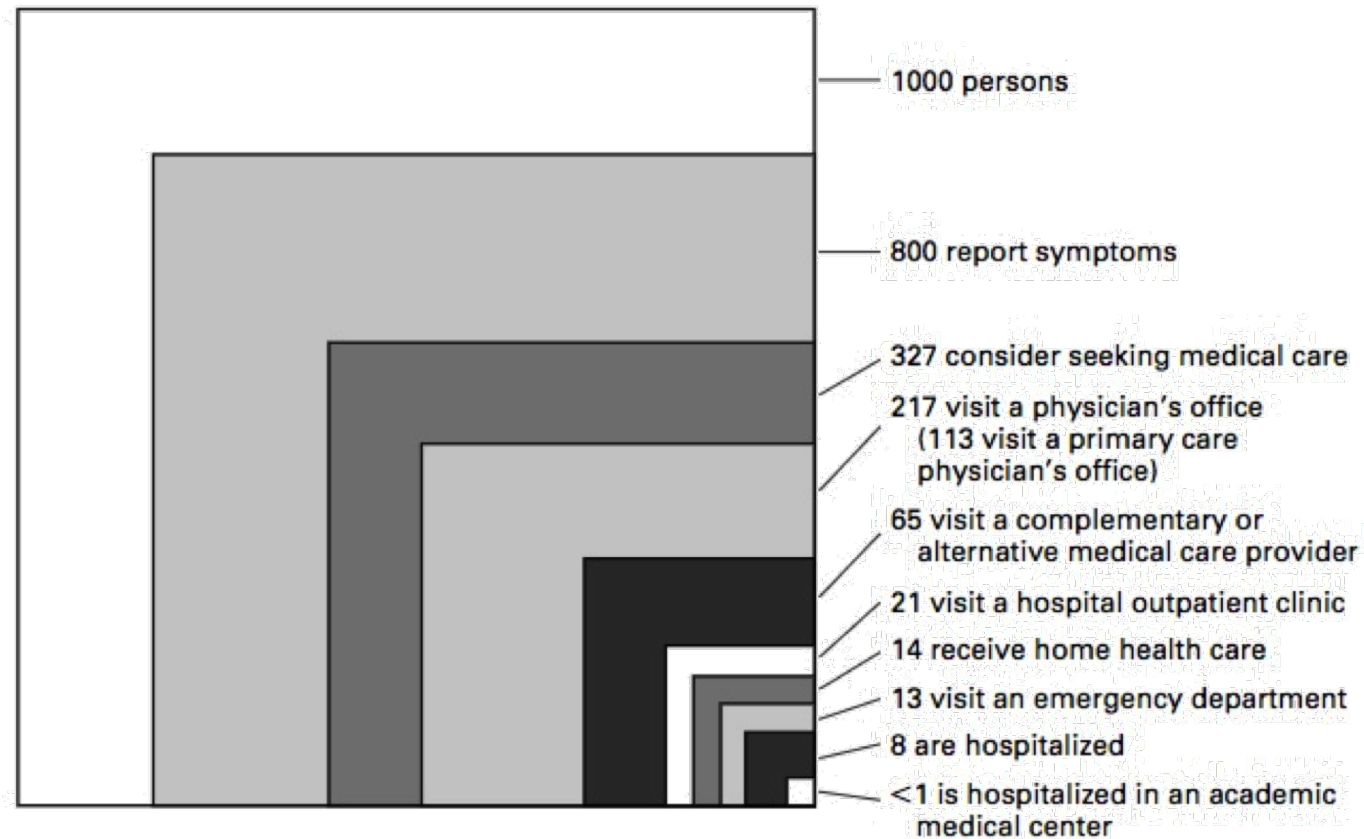
# Background

- 11 of these 45 studies were performed in Europe and yielded an estimate of 24% (95%CI 16 to 33%) for the prevalence of NAFLD.
- 5 of these 11 studies used imaging techniques to diagnose FL and were performed in the general population (3, 5, 8–10), one being a nested case-control study (3).

# Background

- The so-called “ecology of medical care” model provides a strong rationale to expect that the estimates of illness made in the general population will differ from those obtained in other contexts and this has indeed been repeatedly shown in practice (11, 12).

# Background



**Figure 2.** Results of a Reanalysis of the Monthly Prevalence of Illness in the Community and the Roles of Various Sources of Health Care.

Each box represents a subgroup of the largest box, which comprises 1000 persons. Data are for persons of all ages.

# Background

- The inescapable conclusion is that the real burden attributable to a given disease cannot be estimated without epidemiological data obtained from the general population (11).

# Background

- There is also mounting evidence that within a given level of the ecology of medical care model (12), the individuals actually studied are often not representative of the persons making up that level.
- For instance, the patients enrolled in trials of NAFLD drugs are not representative of those treated in everyday practice (13).

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# Aim

- It was with the aim of providing data on the epidemiology of FL in the general population that we performed the Bagnacavallo Study of liver disease (BCV Study).



## **Aim #1**

- 1) to evaluate the prevalence of and the risk factors for FL in a cross-section of the general population of a Northern Italy town;

## **Aim #2**

- 2) to develop a cohort of subjects from the general population where the association between FL and incident health outcomes could be studied;

## **Aim #3**

- 3) to develop a cohort of subjects from the general population where nested case-control studies of potential risk factors for FL could be performed (taking the advantage of a purposely built serum bank)

# Aim

- The present talk deals with the first aim, that is the estimation of the prevalence of and the risk factors for FL in a general population.

# Aim

## **PREVALENCE OF AND RISK FACTORS FOR FATTY LIVER IN THE GENERAL POPULATION OF NORTHERN ITALY: THE BAGNACAVALLO STUDY**

Francesco Giuseppe FOSCHI <sup>1\*</sup>, Giorgio BEDOGNI <sup>2\*</sup>, Marco DOMENICALI <sup>3</sup>, Pierluigi GIACOMONI <sup>4</sup>, Anna Chiara DALL'AGLIO, Francesca DAZZANI, Arianna LANZI, Fabio CONTI, Sara SAVINI, Gaia SAINI, Mauro BERNARDI, Pietro ANDREONE, Amalia GASTALDELLI, Andrea CASADEI GARDINI, Claudio TIRIBELLI <sup>2\*</sup>, Stefano BELLENTANI, Giuseppe Francesco STEFANINI

\* These Authors contributed equally to the present work.

# Outline

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# Study design

- Cross-sectional study performed between October 2005 and March 2009.
- All the citizens of Bagnacavallo (Ravenna, Emilia-Romagna, Italy) aged 30 to 60 years as of January 2005 were eligible and were invited by written letter to participate to the study.
- Public encounters were also held in order to promote participation into the study.

# Study design

- Altered liver enzymes (ALE) were defined as alanine transaminase (ALT) > 40 U/l and/or AST > 37 U/l.
- The study protocol specified that all ALE+ and at least 50% of ALE- citizens had to undergo liver ultrasonography (LUS).
- The study was approved by the Local Ethical Committee and all citizens gave written informed consent.



# Clinical assessment

- All the citizens underwent a detailed clinical history and physical examination following the model of the Dionysos Study (5, 15).

# Alcohol intake

- Current alcohol intake was assessed retrospectively by trained interviewers by measuring the quantity (grams) of beer, wine and liquor drunk in the week prior to the enrollment (16).
- Such quantity was divided by 7 to obtain a daily estimate and converted into alcohol units with rounding to the next integer.
- The conversion was done using an alcohol unit corresponding to 10 g of ethanol.

# Anthropometry

- Weight and height were measured following international guidelines (17).
- Body mass index (BMI) was calculated and classified following the NIH guidelines (18).
- Waist circumference (WC) was measured at the midpoint between the last rib and the iliac crest (19).

# Laboratory assessment

- 1) glucose;
- 2) triglycerides;
- 3) total cholesterol;
- 4) high-density lipoprotein (HDL) cholesterol;
- 5) low-density lipoprotein (LDL) cholesterol;
- 6) ALT;
- 7) AST;
- 8) gamma-glutamyl-transferase (GGT);
- 9) bilirubin;
- 10) hepatitis B surface antigen (HBsAg);
- 11) antibodies against hepatitis C virus.

# Metabolic syndrome

- The metabolic syndrome (MS) was diagnosed using the harmonized international definition (20).

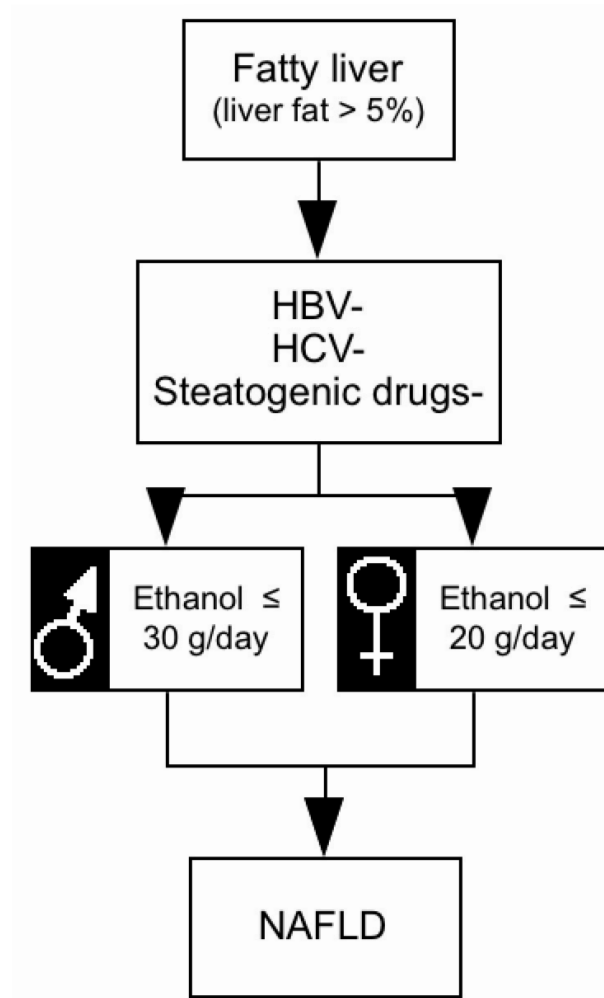
# Metabolic syndrome

- $\geq 3$  of the following (20)
  - Large WC : WC  $\geq 102$  cm in men or  $\geq 88$  cm in women;
  - High triglycerides: triglycerides  $\geq 150$  mg/dl or use of triglyceride-lowering drugs;
  - Low HDL: HDL  $< 40$  mg/dl in men or  $< 50$  mg/dl in women or use of HDL-increasing drugs;
  - High blood pressure: systolic blood pressure  $\geq 130$  mm Hg or diastolic blood pressure  $\geq 85$  mm Hg or use of blood pressure-lowering drugs;
  - High glucose: glucose  $\geq 100$  mg/dl or use of glucose lowering drugs.

# Liver ultrasonography

- LUS was performed by five experienced physicians following international guidelines and using the same methodology of the Dionysos Nutrition and Liver Study (DNL) (21, 22).
- Normal liver / light FL / moderate FL / severe FL

# Diagnosis of NAFLD



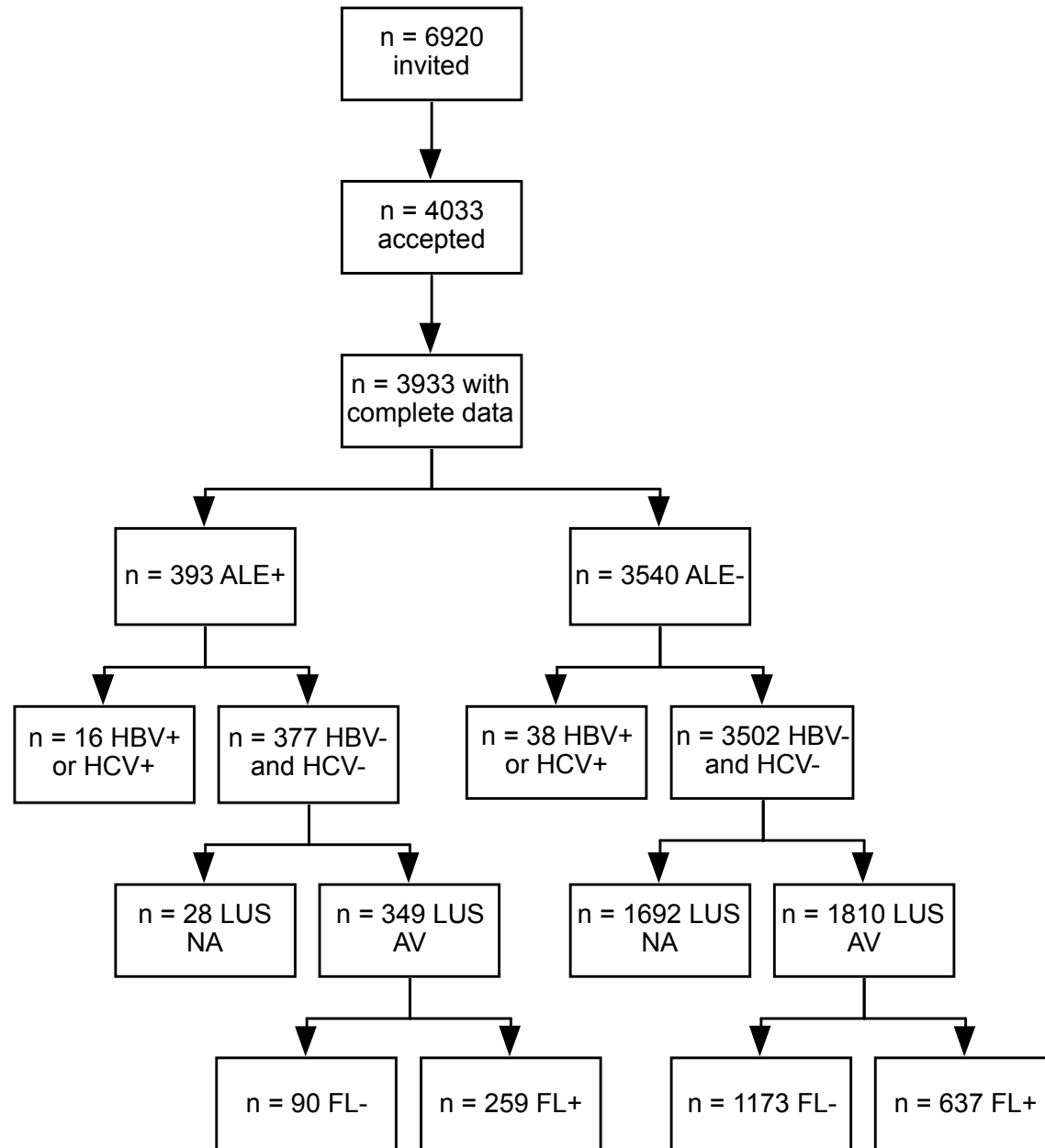


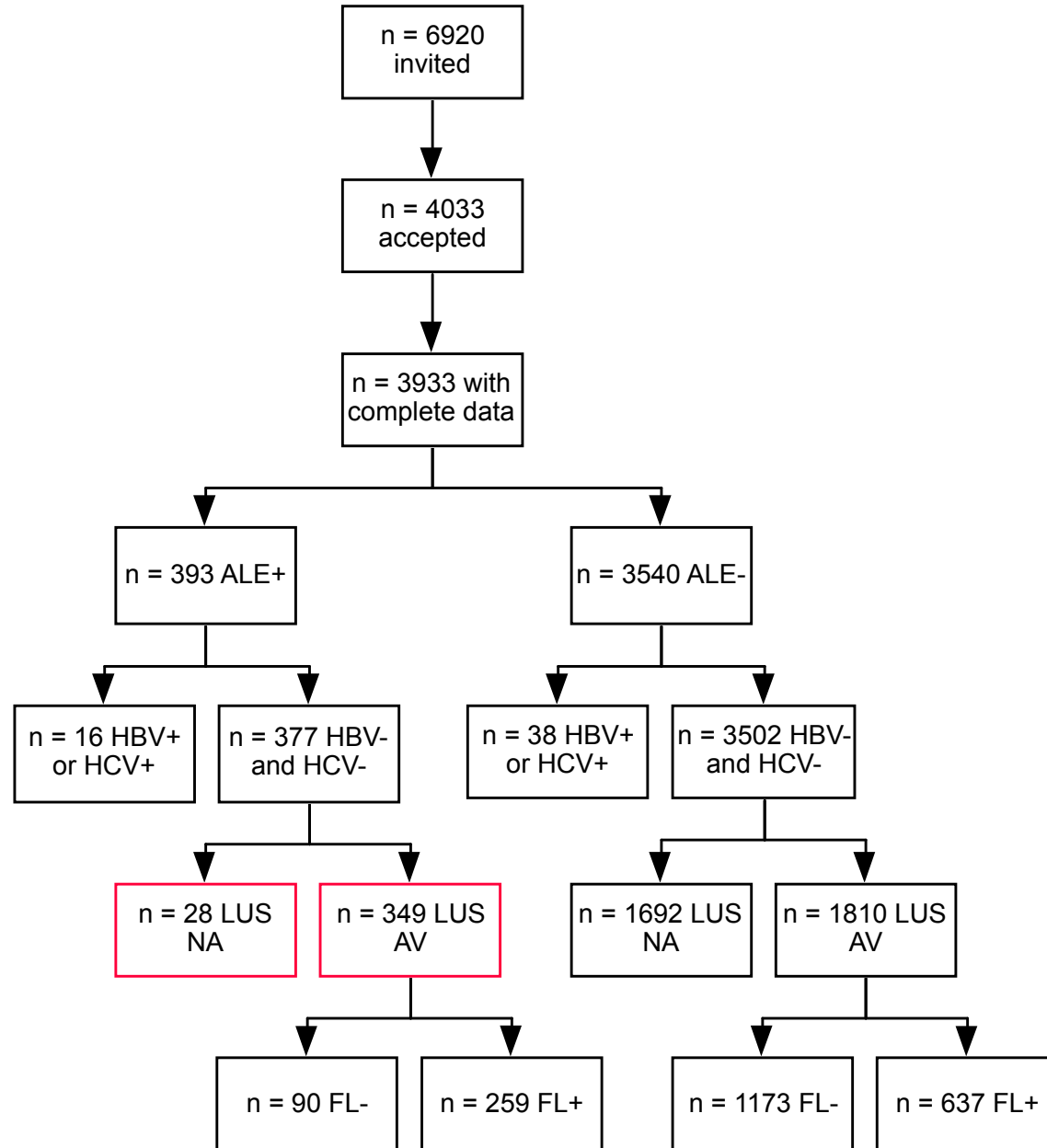
# Statistical analysis

- I will describe it succinctly while discussing the Results

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	ALE+ LUS available <i>n</i> = 349	ALE+ LUS not available <i>n</i> = 28	<i>p</i> -value*
Male sex	267 (76.5%)	25 (89.3%)	0.12
Age (years)	47 (40-55)	45 (40-52)	0.41
Weight (kg)	84.0 (74.0-95.0)	83.0 (75.5-95.0)	1.00
Height (m)	1.73 (1.67-1.79)	1.73 (1.70-1.78)	1.00
BMI (kg/m <sup>2</sup> )	27.9 (25.4-30.9)	27.4 (24.2-29.6)	0.79
Waist circumference (cm)	105.0 (100.0-113.0)	104.0 (97.5-111.5)	0.63
Glucose (mg/dl)	93 (87-102)	93 (89-99)	1.00
Triglycerides (mg/dl)	138 (98-206)	103 (77-166)	0.03
Total cholesterol (mg/dl)	215 (192-240)	216 (182-228)	1.00
HDL cholesterol (mg/dl)	50 (44-61)	53 (46-60)	0.21
LDL cholesterol (mg/dl)	138 (117-159)	125 (106-152)	0.41
Systolic blood pressure (mm Hg)	130 (120-140)	130 (122-140)	1.00
Diastolic blood pressure (mm Hg)	85 (80-90)	82 (80-90)	1.00
ALT (U/l)	50 (44-63)	44 (41-58)	0.04
AST (U/l)	33 (29-41)	32 (27-38)	0.66
GGT (U/l)	42 (27-69)	57 (30-74)	0.02
Total bilirubin (mg/dl)	0.62 (0.49-0.90)	0.60 (0.46-0.79)	0.72
Alcohol intake (units/day)	3 (1-5)	4 (2-5)	1.00

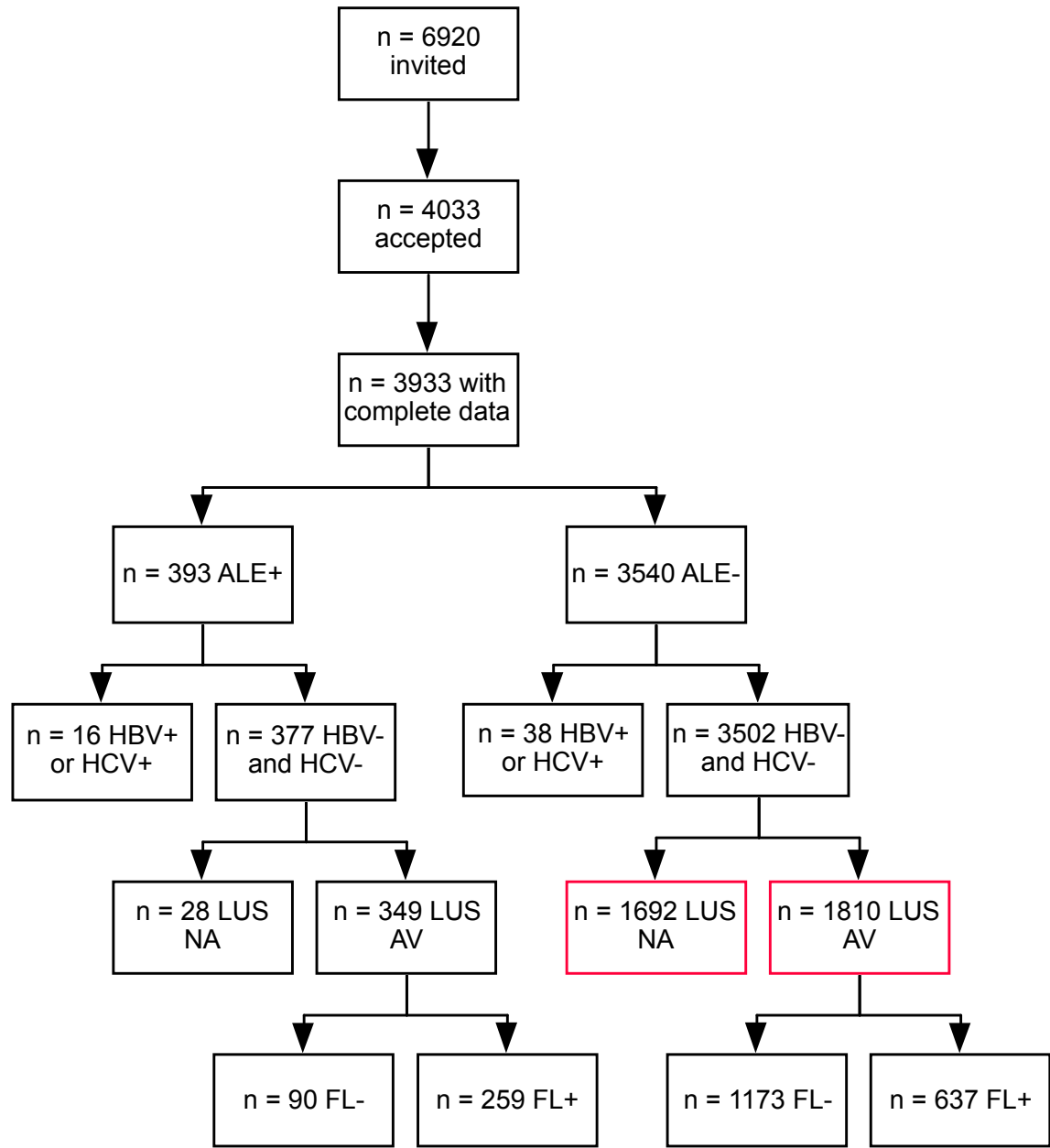
Values are given as median (interquartile range) for continuous variables and as number (proportion) for discrete variables.

\*Median regression for continuous variables and Pearson's Chi-square test for discrete variables.

### Supplementary Table 1

Comparison of the subjects with and without liver ultrasonography among the citizens with altered liver enzymes.

Abbreviations: ALE = altered liver enzymes; LUS = liver ultrasonography; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl transferase.



	ALE- LUS available <i>n</i> = 1810	ALE- LUS not available <i>n</i> = 1692	<i>p</i> -value *
Male sex	812 (44.9%)	701 (41.4%)	0.04
Age (years)	49 (41-56)	47 (40-55)	<0.001
Weight (kg)	72.0 (61.0-82.0)	69.0 (60.0-79.0)	<0.001
Height (m)	1.68 (1.60-1.74)	1.68 (1.60-1.74)	1.00
BMI (kg/m <sup>2</sup> )	25.1 (22.6-28.1)	24.4 (22.1-27.0)	<0.001
Waist circumference (cm)	100.0 (93.0-107.0)	99.0 (92.0-104.0)	0.005
Glucose (mg/dl)	89 (83-96)	87 (82-94)	<0.001
Triglycerides (mg/dl)	97 (68-139)	89 (65-129)	<0.001
Total cholesterol (mg/dl)	207 (184-234)	203 (179-226)	<0.001
HDL cholesterol (mg/dl)	61 (51-73)	62 (52-74)	0.15
LDL cholesterol (mg/dl)	126 (104-150)	122 (101-144)	0.002
Systolic blood pressure (mm Hg)	125 (120-140)	120 (120-130)	<0.001
Diastolic blood pressure (mm Hg)	80 (80-90)	80 (80-90)	1.00
ALT (U/l)	20 (15-26)	19 (15-24)	0.004
AST (U/l)	20 (18-24)	20 (17-23)	1.000
GGT (U/l)	17 (12-26)	15 (11-22)	<0.001
Total bilirubin (mg/dl)	0.60 (0.40-0.81)	0.54 (0.40-0.70)	<0.001
Alcohol intake (units/day)	2 (0-4)	2 (0-4)	1.000

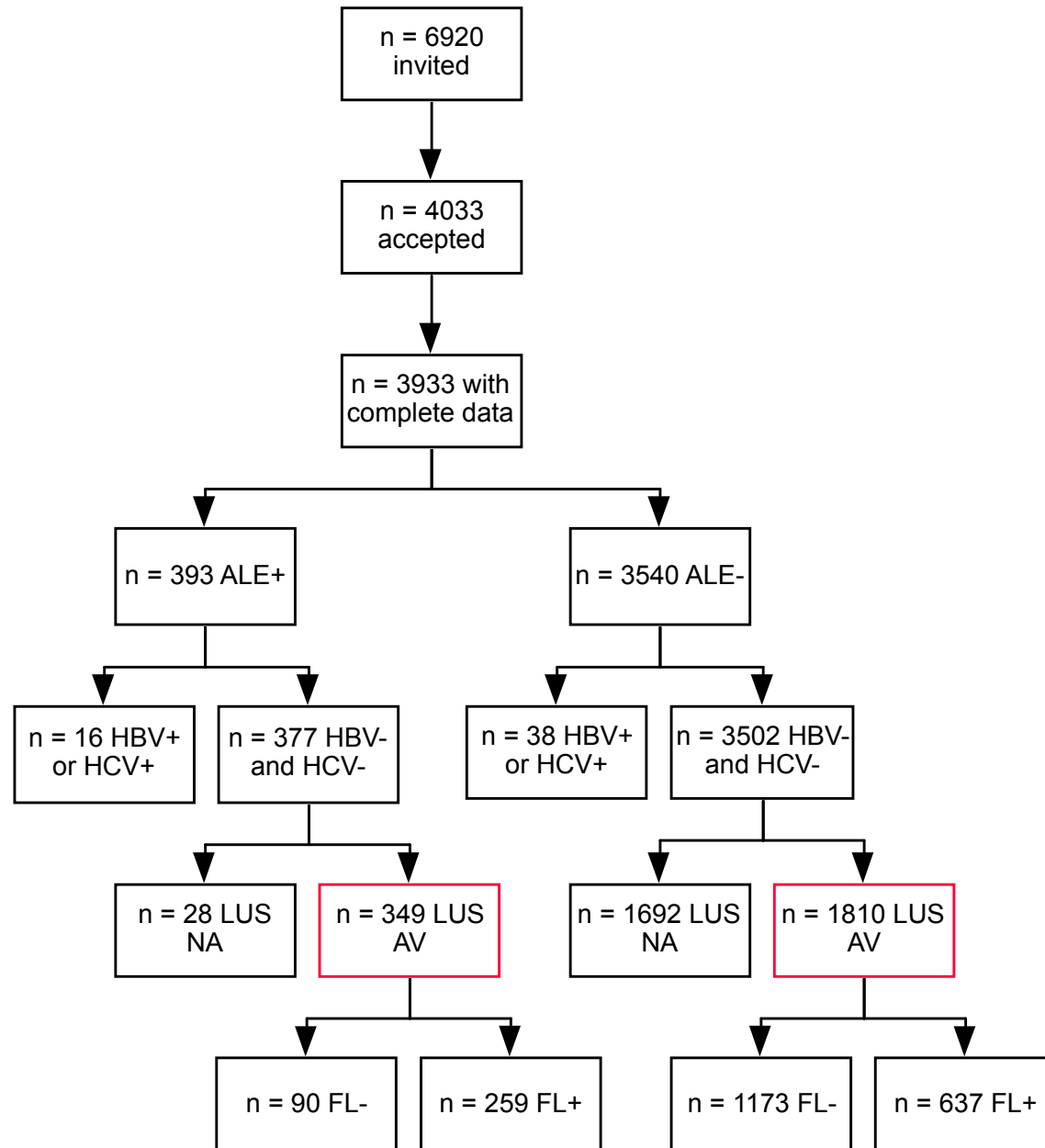
Values are given as median (interquartile range) for continuous variables and as number (proportion) for discrete variables.

\*Median regression for continuous variables and Pearson's Chi-square test for discrete variables.

## Supplementary Table 2

Comparison of the subjects with and without liver ultrasonography among the citizens with normal liver enzymes.

Abbreviations: ALE = altered liver enzymes; LUS = liver ultrasonography; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl transferase.





	ALE- n = 1810	ALE+ n = 349	p-value*
Age (years)	49 (41-56)	47 (40-55)	0.03
Male sex	812 (44.9%)	267 (76.5%)	<0.001
Weight (kg)	72.0 (61.0-82.0)	84.0 (74.0-95.0)	<0.001
Height (m)	1.68 (1.60-1.74)	1.73 (1.67-1.79)	<0.001
BMI (kg/m <sup>2</sup> )	25.1 (22.6-28.1)	27.9 (25.4-30.9)	<0.001
BMI class (NIH)			<0.001
Underweight	19 (1.0%)	0 (0.0%)	
Normal	871 (48.1%)	66 (18.9%)	
Overweight	607 (33.5%)	170 (48.7%)	
Obesity class 1	230 (12.7%)	81 (23.2%)	
Obesity class 2	65 (3.6%)	28 (8.0%)	
Obesity class 3	18 (1.0%)	4 (1.1%)	
Fatty liver	637 (35.2%)	259 (74.2%)	<0.001
Fatty liver degree			<0.001
Light	428 (67.2%)	107 (41.3%)	
Moderate	151 (23.7%)	102 (39.4%)	
Severe	58 (9.1%)	50 (19.3%)	
Waist circumference (cm)	100.0 (93.0-107.0)	105.0 (100.0-113.0)	<0.001
Large waist circumference	1236 (68.3%)	259 (74.2%)	0.028
Glucose (mg/dl)	89 (83-96)	93 (87-102)	<0.001
High fasting glucose	307 (17.0%)	109 (31.2%)	<0.001
Triglycerides (mg/dl)	97 (68-139)	138 (98-206)	<0.001
High triglycerides	405 (22.4%)	159 (45.6%)	<0.001
Total cholesterol (mg/dl)	207 (184-234)	215 (192-240)	0.005
HDL cholesterol (mg/dl)	61 (51-73)	50 (44-61)	<0.001
Low HDL	219 (12.1%)	64 (18.3%)	0.002
LDL cholesterol (mg/dl)	126 (104-150)	138 (117-159)	<0.001
Systolic blood pressure (mm Hg)	125 (120-140)	130 (120-140)	<0.001
Diastolic blood pressure (mm Hg)	80 (80-90)	85 (80-90)	<0.001
High blood pressure	1053 (58.2%)	270 (77.4%)	<0.001
Metabolic syndrome	444 (24.5%)	171 (49.0%)	<0.001
Metabolic syndrome score			<0.001
0	216 (11.9%)	19 (5.4%)	
1	595 (32.9%)	59 (16.9%)	
2	555 (30.7%)	100 (28.7%)	
3	294 (16.2%)	97 (27.8%)	
4	117 (6.5%)	59 (16.9%)	
5	33 (1.8%)	15 (4.3%)	
ALT (U/l)	20 (15-26)	50 (44-63)	<0.001
AST (U/l)	20 (18-24)	33 (29-41)	<0.001
GGT (U/l)	17 (12-26)	42 (27-69)	<0.001
Total bilirubin (mg/dl)	0.60 (0.40-0.81)	0.62 (0.49-0.90)	0.003

Values are given as median (interquartile range) for continuous variables and number (proportion) for dichotomous variables; \*Median regression for continuous variables and Pearson's Chi-square test for binary categorical variables

**Table 1 - Comparison of citizens with and without altered liver enzymes**

Abbreviations: ALE = altered liver enzymes; BMI = body mass index; NAFLD = non-alcoholic fatty liver disease; AFLD = alcoholic fatty liver disease; NIH = National Institutes of Health; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl transferase.

	M1	M2	M3	M4	M5	M6
ALE	5.3** [4.1 to 6.9]	5.1** [3.9 to 6.7]	3.9** [2.9 to 5.2]	4.0** [3.0 to 5.4]	4.2** [3.2 to 5.6]	3.7** [2.8 to 5.0]
Male	—	2.1** [1.7 to 2.5]	2.0** [1.6 to 2.5]	2.1** [1.7 to 2.6]	2.0** [1.6 to 2.5]	2.4** [1.9 to 3.1]
Age (years) / 10	—	1.8** [1.6 to 2.0]	1.6** [1.4 to 1.8]	1.5** [1.4 to 1.7]	1.5** [1.4 to 1.7]	1.4** [1.3 to 1.6]
BMI (kg/m <sup>2</sup> ) / 5	—	—	3.9** [3.3 to 4.5]	—	—	—
Alcohol intake (units)	—	—	1.0 [0.9 to 1.0]	1.0 [1.0 to 1.1]	1.0 [0.9 to 1.0]	1.0 [0.9 to 1.0]
Waist circumference (cm) / 10	—	—	—	2.5** [2.3 to 2.8]	—	—
Metabolic syndrome	—	—	—	—	5.1** [4.1 to 6.3]	—
Large waist circumference	—	—	—	—	—	2.9** [2.3 to 3.8]
High triglycerides	—	—	—	—	—	3.1** [2.4 to 3.9]
Low HDL	—	—	—	—	—	1.6* [1.2 to 2.2]
High blood pressure	—	—	—	—	—	1.9** [1.5 to 2.3]
High glucose	—	—	—	—	—	2.0** [1.5 to 2.6]
<i>n</i>	2159	2159	2159	2159	2159	2159
AIC	2750	2595	2131	2244	2376	2266
BIC	2762	2618	2165	2278	2405	2317
ROC-AUC	0.61	0.72	0.83	0.81	0.79	0.81
Pseudo-R <sup>2</sup> (Nagelkerke)	0.11	0.20	0.42	0.37	0.31	0.36

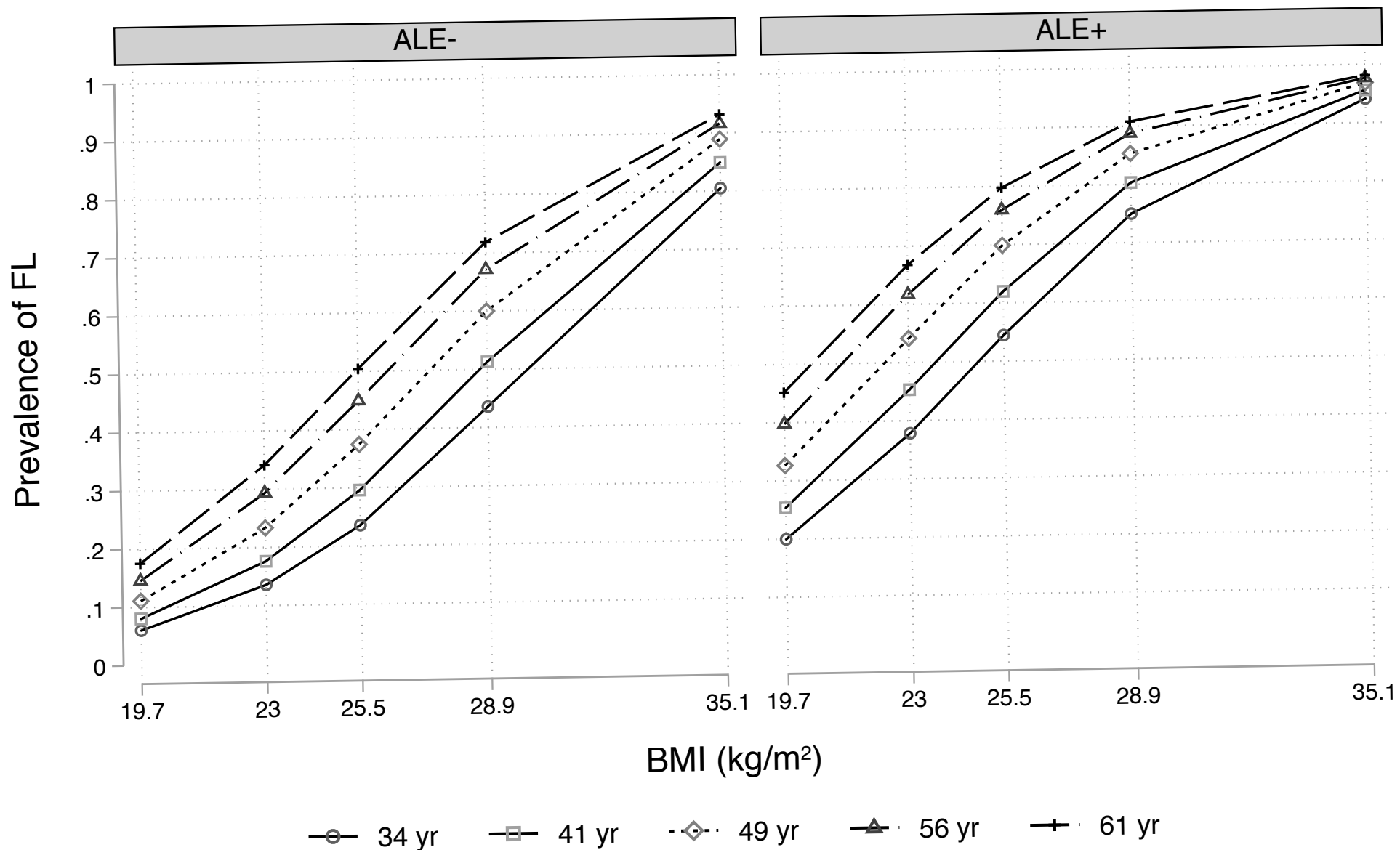
\* $p < 0.01$ ; \*\*  $p < 0.001$

## Table 2

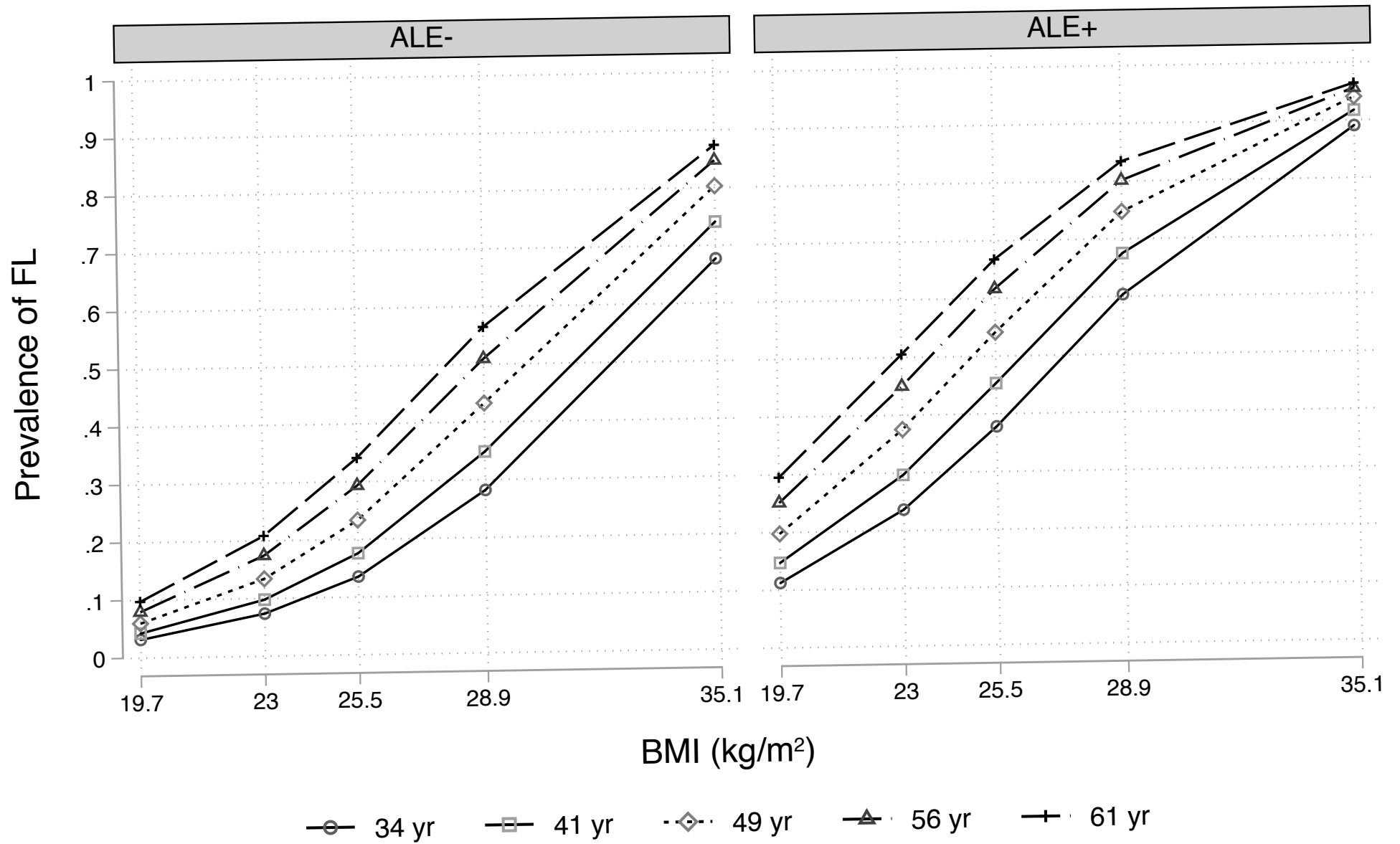
Logistic regression models used to investigate the association between fatty liver and potential risk factors. Values are odds ratios and 95% confidence intervals.

Abbreviations: M# = model number; ALE = altered liver enzymes; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; AIC = Akaike information criterion; BIC = Bayesian information criterion; ROC-AUC = area under the ROC curve; R<sup>2</sup> = squared R for logistic regression.

# Men



# Women



	ALE+	ALE-
Normal liver	0.26 (0.21 to 0.31)	0.65 (0.63 to 0.67)
NAFLD	0.46 (0.41 to 0.51)	0.22 (0.21 to 0.24)
ALFD	0.28 (0.24 to 0.33)	0.13 (0.11 to 0.14)

Prevalence of normal liver, NAFLD and AFLD. Values are proportions and 95% confidence intervals.

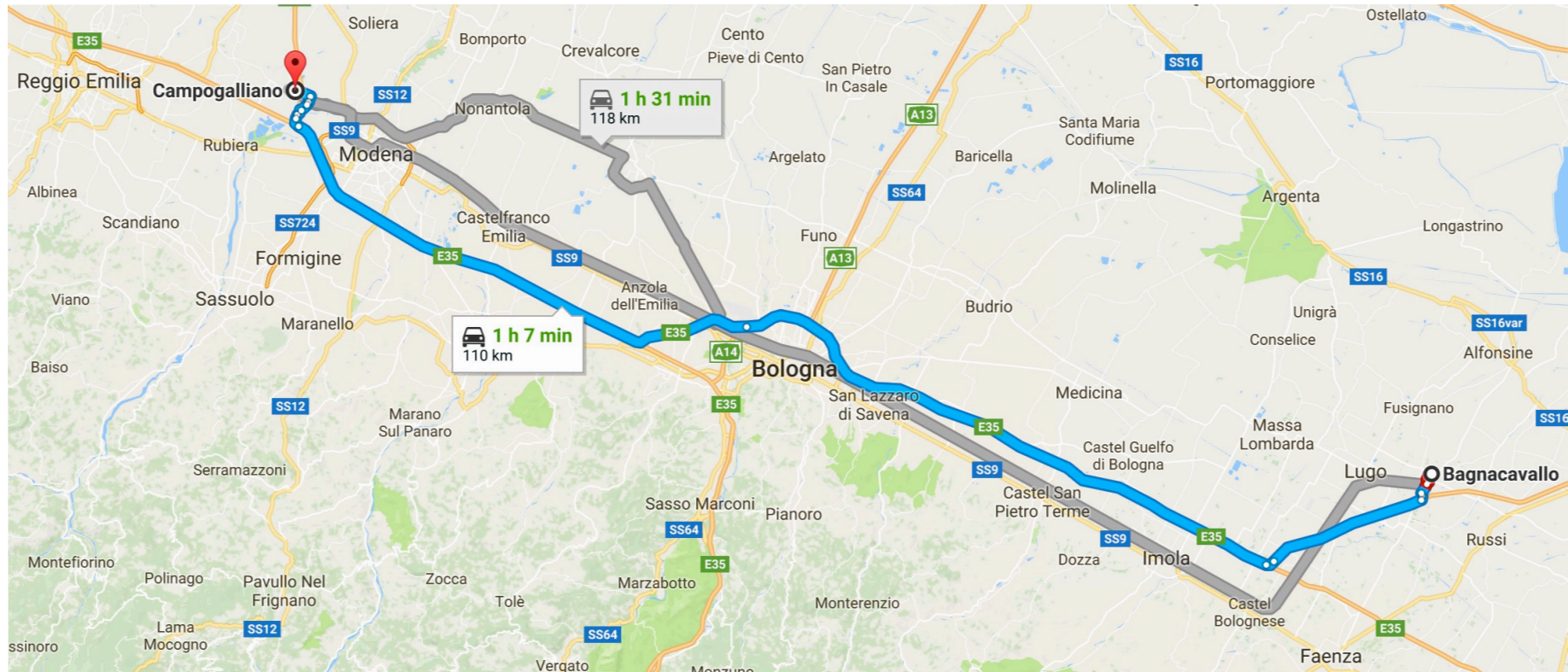
# Outline

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# Discussion

- 74% of ALE+ citizens had FL compared to 35% of ALE- citizens.

# Discussion





# Discussion

- In the Dionysos Nutrition & Liver (DNL) Study, performed in the same region of Northern Italy during 2002/3, 44% of SLD+ citizens had FL compared to 35% of SLD- citizens (5).

# Discussion

- The BCV study and DNL estimates are unfortunately not comparable because of the different operational definitions of ALE and SLD.
- The SLD criteria did in fact include an altered GGT (> 35 U/l), did not consider AST, and did consider “altered” an ALT > 30 U/l (5).
- The BCV Study confirms nonetheless the DNL finding that FL is quite common (35%) among adults citizens with normal liver enzymes.

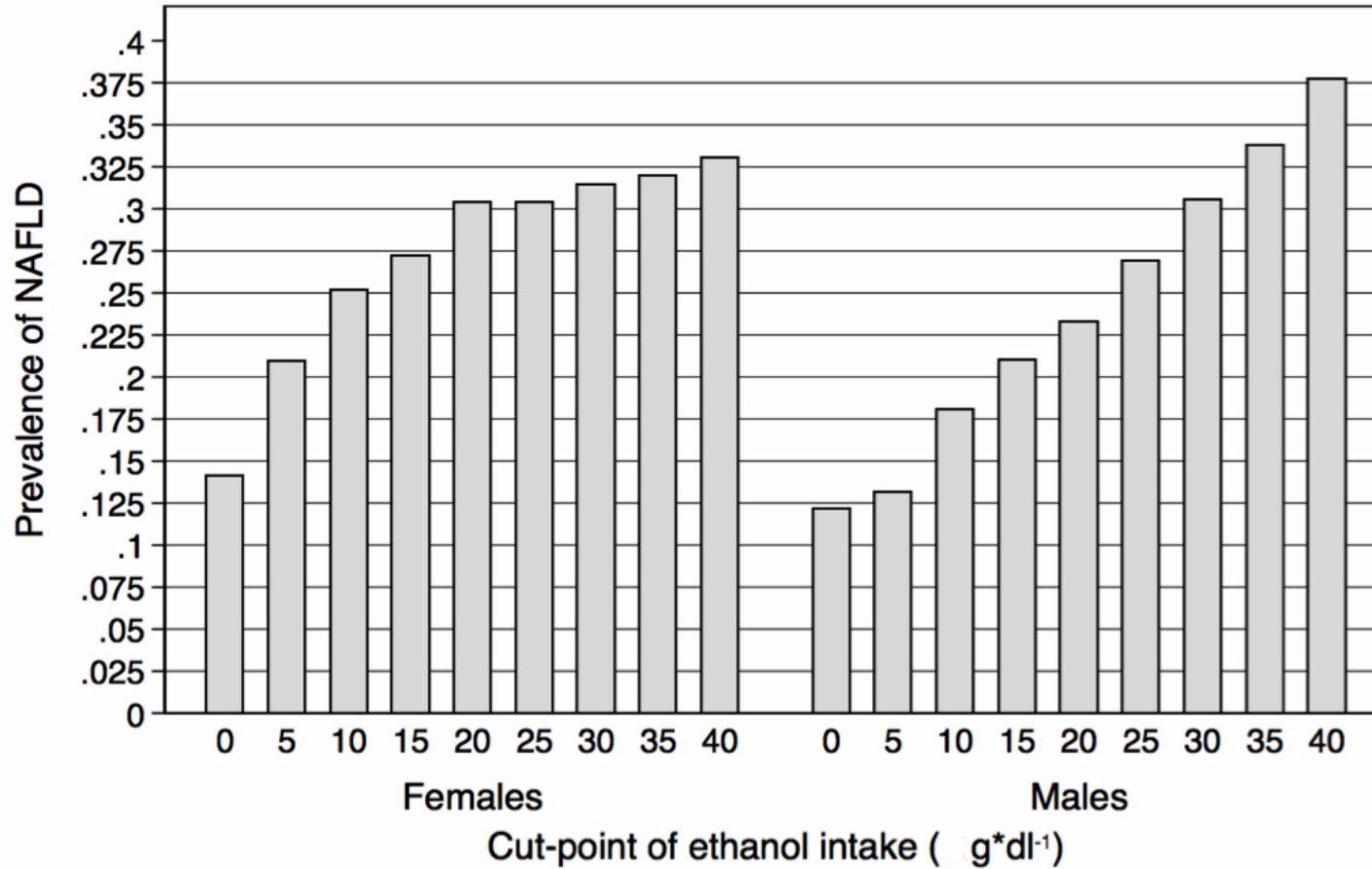
# Discussion

- The prevalence of NAFLD was 46% among ALE+ and 22% among ALE- citizens.
- The corresponding figures for SLD+ and SLD- citizens in the DNL Study were 25% and 20% (5).
- The BCV and DNL estimates are unfortunately not comparable not only because of the different operational definitions of ALE and SLD but also because the DNL study employed a different cut-point of alcohol intake to diagnose NAFLD in men ( $\leq 20$  g/day) and used a 7-day prospective diary to measure ethanol intake (5).

# Discussion

- We have previously shown that small errors in the estimation of ethanol intake may have a substantial impact on the estimated prevalence of NAFLD vs. AFLD (5, 31).
- This is one of the reasons why we choose to focus on FL instead of NAFLD vs. AFLD for the present analysis (5, 31).

# Discussion



Calculated from Bedogni G. Hepatology. 2005;42:44

# Discussion

- The analysis of the risk factors for FL yielded two very interesting findings.

# Discussion

- The first finding is that ethanol intake was not an independent predictor of FL in the general population.
- The DNL Study gave the same finding even if a direct comparison of the DNL and BCV studies is not possible because of the different instruments used to measure alcohol intake (22).

## Discussion

- The second finding is that all the components of the MS were associated to FL independently of ALE status, gender, age and alcohol intake.
- Moreover, the MS components together performed better than MS alone at identifying the presence of FL.



# Discussion

- The dichotomization implicit in the concept of MS has been criticized by research methodologists on the basis of both clinical and statistical grounds (31).
- The findings of the present study offers a further empirical argument for preferring the use of the single components of the MS instead of the whole MS for the study of the association of FL with cardiometabolic risk factors.

# Strenghts

- The strenghts of the present study are that it was performed in a representative sample of the general population, that it enrolled an high number of subjects, and that it built a serum bank that we plan to use in further studies.

# Limitations

- The most important limitation of the present study is the suboptimal response rate (58%).
- Although this response rate is exactly the same of the DNL Study (31) and is higher than that reported by most studies (32), it is possible that the citizens who refused to participate to the BCV Study differed systematically from those who accepted with an ensuing selection bias.

# Conclusion

- FL was highly prevalent in a Northern Italy town in 2005/9 and was more common among ALE+ citizens.
- It was not associated with alcohol intake but was strongly associated with anthropometry and the MS components.

## Looking ahead

- The cross-sectional data presented in this talk will inform the ongoing and future analyses of the BCV cohort, which we hope will offer new and relevant information on the burden of FL in the general population.

**Thank you!**

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