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Original Articles

Cutoff Point Separating Affected and Unaffected Familial Hypercholesterolemic Patients Validated by LDL-receptor Gene Mutants

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Familial hypercholesterolemia (FH) results from low-density lipoprotein (LDL) receptor gene mutations. Heterozygotes have twice normal LDL-cholesterol concentrations in early childhood, and experience early myocardial infarction. We demonstrated bimodal cholesterol frequency distributions, independently confirming existence of an identifiable hypercholesterolemic subpopulation. We assayed blood lipids in 181 FH patients genetically diagnosed and 100 unaffected relatives. Receiver operating characteristics curves were constructed. Total cholesterol and LDL-cholesterol concentrations showed bimodality. A total cholesterol cutoff of 225 mg/dl produced results agreeing with DNA testing (specificity, 98.5%; sensitivity, 99.4%). An LDL-cholesterol cutoff of 161–163 mg/dl produced 98.5% specificity and 98.3% sensitivity. Areas under curves were 0.9826 ± 0.0058 for total cholesterol, and 0.9852 ± 0.0043 for LDL-cholesterol. In conclusion, we define total cholesterol and LDL-cholesterol levels of 225 and 160 mg/dl, respectively, as cutoff points of normal subjects and FH patients. ***J Atheroscler Thromb, 2005; 12: 35–40.***

Keywords: Familial hypercholesterolemia, LDL-cholesterol, Cuttoff point, Receiver operating characteristics analysis

Introduction

Hypercholesterolemia is a major coronary risk factor and many epidemiologic studies have linked high serum cholesterol to higher incidence of coronary heart disease (CHD) (1). In the second report from the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP II) a cutoff point defining a high blood cholesterol (240 mg/dl) marked a steep rise in risk for CHD. This value corresponded to approximately the 80th percentile in the third adult US population National Health and Nutrition Examination Survey (NHANES III) (2). Yet a precise definition of hypercholesterolemia is difficult to establish. Often an abnormally high laboratory variable is considered to be the value defining the upper 5% of the population (the 95th percentile). Frequency distributions of serum cholesterol concentrations have been reported to be continuous and unimodal in the general population, with no clear point of separation between individuals with normal and abnormally high values. Serum cholesterol concentrations depend upon both genetic and environmental factors, and the mean ± standard deviation (SD) in "healthy subjects" has been used to define the normal range. However, use of this definition is unreasonable, since CHD is a major cause

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of death in highly industrialized countries where much of the population has excessive blood cholesterol levels.

Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations of the LDL receptor gene (3). FH heterozygotes cause approximately double the normal LDL cholesterol concentration in early childhood, and have increased rise of early myocardial infarction (4, 5). Serum total cholesterol concentrations in unaffected subjects, FH heterozygotes and FH homozygotes showed a distinct trimodal distribution (4). Thus, the bimodal frequency distribution of cholesterol concentration in unaffected and heterozygous FH patients can be used to separate the general population into normal and hypercholesterolemic groups because of increased low-density lipoprotein (LDL)-cholesterol concentrations.

The present study demonstrates that serum total cholesterol concentrations were distributed bimodally in FH families studied to determine LDL-receptor gene abnormalities (6). The observation of bimodality in cholesterol frequency distributions may provide an independent confirmation of a hypercholesterolemic subgroup within the general population.

Methods

Subjects

Subjects included 181 FH patients and 100 unaffected first- and second-degree relatives. No subjects were taking lipid-lowering drugs, and none had a disease affecting serum lipid concentrations. All FH patients were heterozygous and were diagnosed by abnormalities of the LDL-receptor gene, while unaffected family members showed no LDL receptor gene mutations (6).

Lipid measurements

Blood samples were drawn for assays after overnight fasting. Concentrations of serum total cholesterol, triglyceride (TG), and HDL-cholesterol were determined enzymatically. LDL-cholesterol concentrations were derived using the Friedewald formula (7).

Molecular analysis

Genomic DNA was isolated according to a standard method from the buffy coat of a centrifuged 5-ml blood sample anticoagulated with disodium EDTA. Techniques used for PCR-denaturing gradient gel electrophoresis (DGGE), DNA sequencing, and Southern blot analysis were reported in our previous paper (6). Briefly, fragments that showed a variant by DGGE were amplified by PCR, and the PCR products were sequenced by an ABI 310 automated sequencer.

Statistical analysis

Lipid concentrations and other parameters were compared between FH and non-FH groups using Student's *t*-test.

Receiver operating characteristics (ROC) analysis

Serum total cholesterol, HDL-cholesterol, and LDL-cholesterol concentrations showed a symmetric normal distribution, while serum triglyceride concentration showed a skewed distribution and was transformed to a logarithmic value (log TG) that showed a normal distribution. Bimodal distributions of total cholesterol and LDL-cholesterol concentrations suggested points that could discriminate FH and non-FH patients. ROC analyses and plotting were performed using a program (ROCKIT) for the Macintosh personal computer (8, 9). To yield a full spectrum of sensitivity/specificity pairs corresponding to all possible decision levels for total and LDL-cholesterol concentrations; these were tested for ability to discriminate FH from non-FH subjects. The ROC curve was constructed by plotting sensitivity on the y-axis against the false-positive fraction (1-specificity) on the x-axis. Areas under ROC curves ranged from 1.0, corresponding to perfect discrimination (upper left corner), to 0.5 (no discrimination) (10, 11).

Results

Clinical features

The number of patients with each LDL-receptor gene mutation is shown in Table 1. The most common was at exon 17 K790X (6). Numbers of male and female subjects were nearly equal. Serum total cholesterol and LDL-cholesterol concentrations in FH respectively were 1.8 and 2.3 times higher than those in non-FH subjects (Table 2). HDL-cholesterol concentrations in FH patients were 10 mg/dl lower than those in non-FH subjects (Table 2). The histogram of serum total cholesterol concentrations in FH and non-FH subjects showed a bimodal distribution (Fig. 1). As for distributions of serum total cholesterol, LDL-cholesterol, HDL-cholesterol, and log TG (Fig. 2), total cholesterol and LDL-cholesterol showed distinct bimodality, while HDL-cholesterol and log TG levels did not.

ROC analysis

Sensitivity and specificity of the criteria proposed above were tested by ROC analysis of a sample of 281 sequentially sampled first- and second-degree relatives in which diagnosis of FH was established using genetic markers (Fig. 3). The proposed total cholesterol criteria of 224 and 225 mg/dl were in agreement with DNA marker, resulting in an observed specificity of 98.5% and sensitivity of 99.4%. The area under the curve was 0.9826 ± 0.0058 for total-cholesterol. LDL-cholesterol cutoffs of 161 to 163 mg/dl produced an observed specificity of

Table 1. Numbers of patients and serum lipid concentrations for each LDL-receptor gene mutation.

LDL-receptor gene mutation	No.	TC		TG		HDL-C		LDL-C	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tonami-1	17	322	58	110	49	48.4	12.5	252	56
Tonami-2	14	316	61	122	100	47.8	12.9	244	60
Okayama	2	346	118	92	21	48.5	5.0	279	118
Exon 2 C25Y TGC-TAC	3	276	49	109	35	26.3	14.6	228	59
Exon 4 R94H CGC-CAC	2	300	1	124	2	54.5	10.6	221	12
Exon 9 D412H GAC-CAC	4	366	67	101	61	46.5	10.7	299	51
Exon 11 FsN543 Cins1689	2	305	59	207	152	57.0	21.0	224	54
Exon 13 1927 ATC 3bp deletion	8	335	42	139	42	42.3	9.4	265	44
Exon 14 P664L CCG-CTG	19	358	46	162	149	38.3	12.4	287	56
Intron 15 2312-3 C-A	17	334	64	126	65	42.0	6.9	267	61
Exon 17 K790X	85	335	56	148	107	48.0	28.0	257	62
Others	8	336	67	128	62	43.0	10.1	267	69

TC: total cholesterol concentration (mg/dl), TG: triglyceride concentration (mg/dl), HDL-C: HDL-cholesterol concentration (mg/dl), LDL-C: LDL-cholesterol concentration (mg/dl)

Table 2. Clinical features and lipid parameters in subjects with and without FH.

	F/M		T-CHOL (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	Age(yrs)
FH	90/91	Mean	331.9	233.3	44.2	260.8	41.9
		SD	57.7	97.6	13.4	58.6	16.7
Non-FH	49/51	Mean	184.8	97.9	53.7	114.8	35.1
		SD	24.6	58.5	13.3	23.8	17.7
		P value	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	

FH: familial hypercholesterolemia, F/M: female/male, T-CHOL: Total cholesterol, TG: triglyceride, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, SD: standard deviation

98.5% and a sensitivity of 98.3%. The area under the fitted curve ± SD was 0.9852 ± 0.0043 for LDL-cholesterol. One of 181 FH patients showed a total cholesterol level less than 225 mg/dl, and none of non-FH patients showed total cholesterol levels higher than 225 mg/dl. Three of 181 FH patients showed LDL-cholesterol levels less than 160 mg/dl and none of the non-FH patients showed LDL-cholesterol levels higher than 160 mg/dl. Thus, an LDL-cholesterol concentration of 160 mg/dl and a total cholesterol concentration of 225 mg/dl had the best ability to discriminate between subjects with and without FH.

Discussion

Premature CHD can result from elevated LDL-cholesterol in blood even in the absence of other risk factors. A striking example is provided by young patients who have

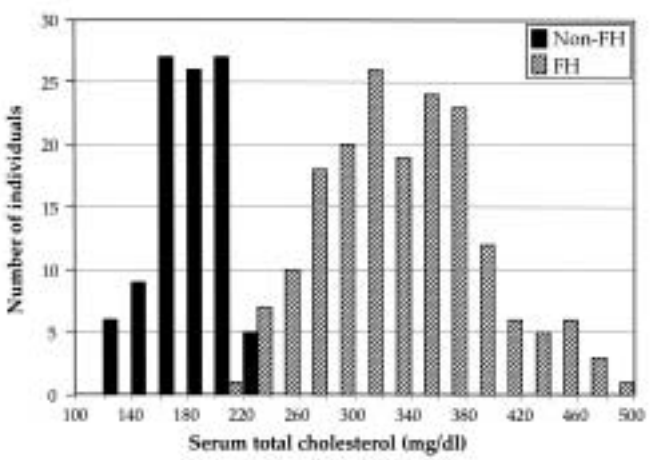

Fig. 1 Histogram of serum cholesterol concentrations in FH (n = 181) and non-FH subjects (n = 100)

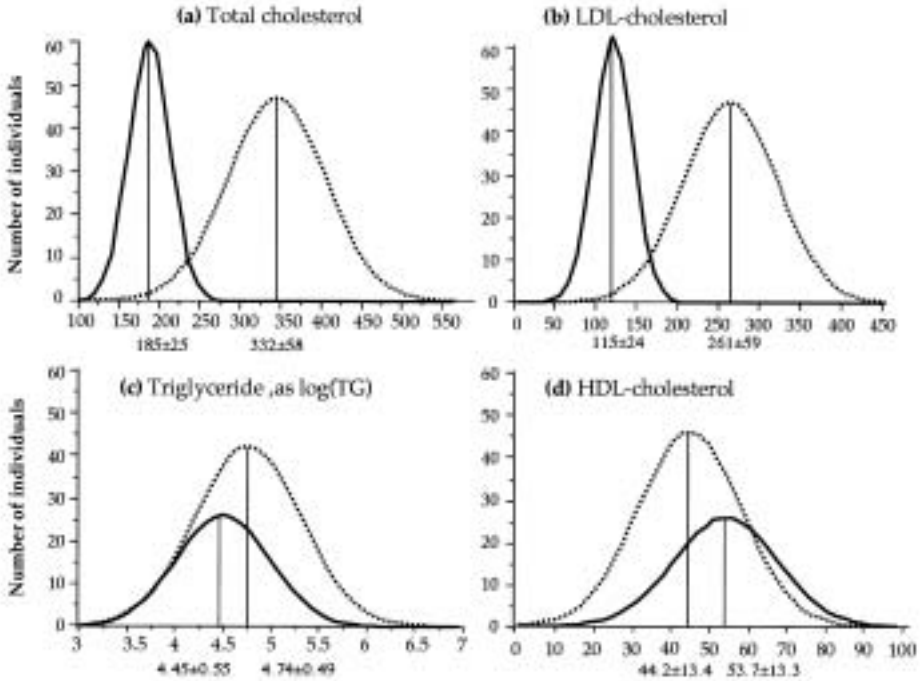

Fig. 2 Distributions of (a) serum total cholesterol, (b) LDL-cholesterol, (c) log triglyceride, and (d) HDL-cholesterol in normal subjects (solid line) and heterozygous familial hypercholesterolemia patients (dotted line). Numbers along the horizontal axis represent mg/dl. Mean ± SD are shown in each subject group.

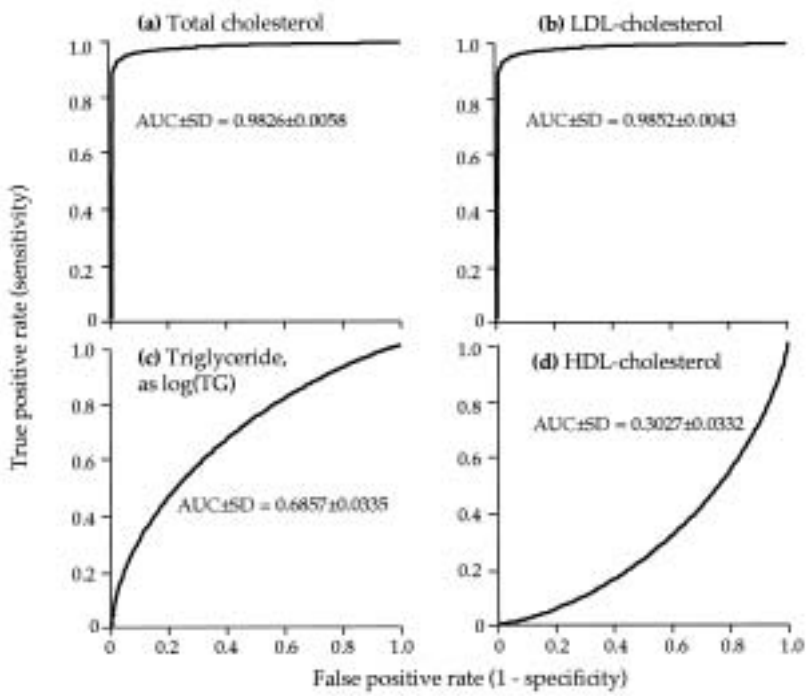

Fig. 3 Receiver operating characteristic (ROC) curves showing discrimination between FH and non-FH subjects by (a) serum total cholesterol, (b) LDL-cholesterol, (c) log triglyceride, and (d) HDL-cholesterol. Areas under ROC curves (AUC) are shown as mean ± SD.

the homozygous form of FH, a rare disorder characterized by essentially complete absence of specific cell-surface receptors that normally remove LDL from the circulation (3). The consequence is an increase in blood cholesterol occurring predominantly in the LDL fraction. As a result LDL cholesterol concentrations are extremely high (500 to 1,000 mg/dl), and severe atherosclerosis and CHD often develop during the first decades of life (12). Patients with the more common heterozygous form of FH have half the normal number of functioning LDL receptors; they have approximately twice-normal LDL-cholesterol concentrations and commonly develop CHD in the middle decades of life (5). To determine the molecular basis of FH in Japan, 200 unrelated patients with clinically diagnosed heterozygous FH were screened for mutations in the coding and promoter regions of the LDL receptor gene (6). Thirty-seven different mutations in the LDL receptor gene were identified in 125 of the patients (62.5%) (6). In the present study the family members of heterozygous FH patients with definite LDL receptor gene mutations were examined. Although most unselected populations show a unimodal distribution of serum cholesterol skewed toward higher concentrations, a bimodal pattern was observed in FH families. The lower component characterized the distribution in individuals with normocholesterolemia, while the upper component characterized the distribution in individuals with hypercholesterolemia. A blood cholesterol concentration of approximately 225 mg/dl divided lower and upper components in the present study. Several reports have outlined methods of screening for FH patients. Umans-Eckenhausen et al. found that the best available cutoff point to diagnose FH by total cholesterol concentration in relatives of known FH patients was the 90th percentile (13, 14).

Clinical accuracy, defined as the ability to discriminate between states of health, is the fundamental property required for any diagnostic test or system, being readily expressed as clinical sensitivity and specificity and represented in an elegant manner by the ROC curve (8). The area under the curve represents the discriminating ability of the particular screening method. Swets suggested the following guidelines for interpreting areas: 0.5 to 0.7, rather low accuracy; 0.7 to 0.9, accuracy useful for some purposes; and greater than 0.9, rather high accuracy (10). The MedPed group compared sensitivity and specificity of differing cutoff values of total serum cholesterol in both a general population sample and in close relatives of confirmed FH patients (15). Wiegman *et al*. (16) reported that by ROC curve analysis of plasma LDL-cholesterol, the largest area was found under the curve of plasma LDL-cholesterol, and the best LDL-cholesterol value for diagnosis of FH in children was 135 mg/dl. To demonstrate the use of ROC curves, Zweig *et al*. reexamined a study of the ability of serum lipid and apolipoprotein measures to discriminate among degrees of CHD in patients undergoing coronary angiography (11). In this study, one of 181 FH patients (0.6%) showed a false negative study by the total cholesterol concentration criteria of 225 mg/dl, and three of 181 FH patients (1.7%) showed false negative study in LDL-cholesterol criteria of 160 mg/dl.

Few diagnostic criteria of hypercholesterolemia have been established, although target concentrations of total cholesterol and LDL-cholesterol have been defined for primary and secondary prevention of CHD in the NCEP ATP III (17) and recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention (18). In NCEP ATP III as well as in ATP II, total cholesterol concentrations below 200 mg/dl are classified as desirable, those from 200 to 239 mg/dl as borderline-high, and those over 240 mg/dl as high. Because the relationship between serum cholesterol and CHD risk shows a continuous, steadily increasing curve, these cutoff points are somewhat arbitrary (like those for blood pressure) (1). At the cutoff point of 240 mg/dl for total cholesterol, CHD risk is roughly double that at 200 mg/dl, and continues to rise steeply (1).

Current diagnostic criteria for diabetes are based on the mean plus 2SD of glucose concentrations after an oral glucose load is given to healthy subjects. Epidemiologic surveys of populations with a high prevalence of type 2 diabetes such as Pima Indians have demonstrated bimodality of 2-h postprandial plasma glucose concentrations (19). Bimodal curve for 2-h plasma glucose after oral glucose loading is used for diagnosis of diabetes with the cutoff at 200 mg/dl (20). Similarly, the bimodality of genetic hypercholesterolemia can be used for diagnosis of hypercholesterolemia. Plasma cholesterol concentrations are distributed over a continuum in a general population, but an approximate threshold separates subjects at substantially increased risk for certain adverse outcomes caused by hypercholesterolemia (e.g. CHD) from those who are not. The relationship between these concentrations and risk of CHD is continuous with no single threshold value separating “normal” from “abnormal” (1). Ideally, diagnostic criteria should not be based on artificial dichotomization of a continuous variable. Serum cholesterol in FH patients correlates highly with LDL-cholesterol resulting from lack of functional LDL receptors. FH, therefore, is a model of pathologically abnormal LDL metabolism, resulting in frequent occurrence of CHD. Discovery of a bimodal distribution of plasma cholesterol concentrations in populations or FH family members strengthened the concept of the hypercholesterolemic state as a distinct clinical entity (4). As serum cholesterol is determined by both genetic and environmental factors, we can use serum cholesterol data determined by genetic factors interacting with the same environmental circumstances underlying diagnostic cri-

teria for hypercholesterolemia in the general population.

In conclusion, we define total cholesterol and LDL-cholesterol concentrations of 225 and 160 mg/dl respectively as cutoff points between normal subjects and FH patients. These cutoff points can be used as diagnostic criteria for hypercholesterolemia in a general Japanese population.

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