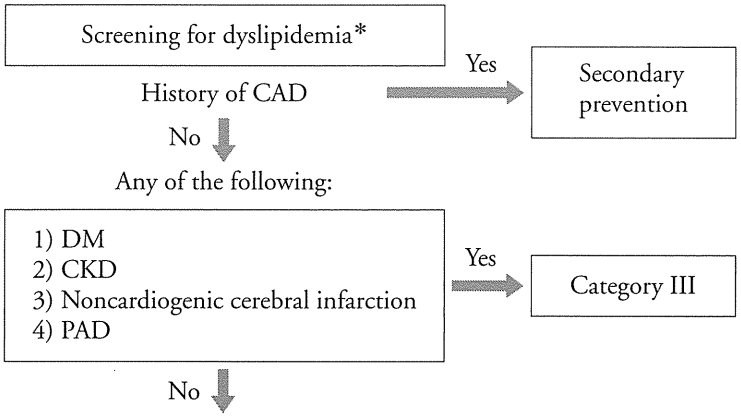


Management categories based on absolute risk for the primary prevention of CAD (For absolute risk, refer to Fig. 2 in Committee Report 1)

| 10-year probability (absolute risk) of CAD death derived from NIPPON DATA80 | Additional risk factors | |
|---|---|---|
| | No additional risk factors | One or more of the following:<br>(1) Hypo-HDL cholesterolemia (HDL-C <40 mg/dL)<br>(2) Family history of premature CAD in first-degree relatives (a man aged <55 years or a women aged <65 years)<br>(3) Impaired glucose tolerance |
| < 0.5% | Category I | Category II |
| ≥0.5% < 2.0% | Category II | Category III |
| ≥2.0% | Category III | Category III |

*This flow chart is not applicable to patients with FH.

**Fig. 2.** Flow Chart for Setting Management Targets for LDL-C.

**Table 6.** Lipid Management Targets for Patients with Different Risk Levels

| Therapeutic principle | Management category | Lipid management target (mg/dL) | | | |
|---|---|---|---|---|---|
| | | LDL-C | HDL-C | TG | Non HDL-C |
| Primary prevention<br>Drug therapy should be considered after lifestyle modification | Category I | <160 | ≥40 | <150 | <190 |
| | Category II | <140 | | | <170 |
| | Category III | <120 | | | <150 |
| Secondary prevention<br>Drug therapy should be considered, together with lifestyle modification | History of CAD | <100 | | | <130 |

high-risk patients, such as those with DM and poor glycemic control or organ damage (e.g., retinopathy, nephropathy or PAD) and those receiving secondary prevention, clinicians should aim to ensure achievement of the targets (**Table 4** and **5**).

In patients with secondary hyperlipidemia com-

**Table 7.** Stratification of cerebrovascular/cardiovascular risk in four categories on the basis of (clinic) blood pressure classification and risk strata

| Risk strata (risk factors other than blood pressure) \ Blood pressure classification | High-normal blood pressure 130-139/85-89 mmHg | Grade I hypertension 140-159/90-99 mmHg | Grade II hypertension 160-179/100-109 mmHg | Grade III hypertension ≥180≥110 mmHg |
|---|---|---|---|---|
| Risk stratum-1 (no other risk factors) | No additive risk | Low risk | Moderate risk | High risk |
| Risk stratus-2[a] (one to two risk factors (other than diabetes) or metabolic symdrome)[b] | Moderate risk[c] | Moderate risk | High risk | High risk |
| Risk stratum-3[a] (three or more risk factors, diabetes, CKD, target organ damage/cardiovascular disease) | High risk[c] | High risk | High risk | High risk |

Abbreviation: CKD, chronic kidney disease.
[a]When obesity and dyslipidemia are present in the absence of other risk factors, risk factors other than the blood pressure level are counted as two, and the risk is classified as the risk stratum-2. However, when other risk factors are present, the total of risk factors is calculated as three or more, and the risk is classified as the risk stratum-3.
[b]Metabolic syndrome in risk stratum-2 indicates patients with an abnormal plasma glucose level (an impaired fasting plasma glucose level of 110-125 mg/dL$^{-1}$ and/of impaired glucose tolerance that does not lead to diabetes), or abnormalities in lipid metabolism in addition to a high-normal or higher blood pressure level and abdominal obesity (males: ≥85 cm, females: ≥90 cm).
[c]Treatment in moderate- and high-risk groups with high-normal blood pressure values is based on the algorithm for treatment of hypertension at initial visit. The management of common cardiovascular risks is important here.
(The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). *Hypertens Res* 2009; 32: 3-107)

plicating hypothyroidism or steroid therapy, the treatment of the primary disease should be given priority, and management of lipid abnormalities should be performed according to the individual circumstances and requirements. With respect to transient hypercholesterolemia associated with pregnancy, drug therapy is not needed, in principle.

### *Step 5B: Management Targets for Hypertension*

- *In young or middle-aged patients < 65 years of age, the target office blood pressure should be < 130/85 mmHg (home blood pressure < 125/80 mmHg).*
- *In elderly patients ≥ 65 years of age, the target office blood pressure should be < 140/90 mmHg (home blood pressure < 135/85 mmHg).*
- *In patients with DM, CKD or a history of MI, the target office blood pressure should be < 130/80 mmHg (home blood pressure < 125/75 mmHg).*
- *In patients with cerebrovascular disease, the target office blood pressure should be < 140/90 mmHg (home blood pressure < 135/85 mmHg).*

Although this section describes the target office blood pressure (**Fig. 1** and **Table 7**), home blood pressure measurements are essential for diagnosing masked hypertension and/or white coat hypertension and diagnosing and treating refractory hypertension and are of equal or greater clinical value than office blood pressure measurements. Home blood pressure measurement is not only useful for assessing hypotensive effects, but also preventing complications due to excessive decreases in blood pressure. Home blood pressure values are generally lower than casual blood pressure values measured in outpatient clinics or during health screenings; thus, the target home blood pressure is lower than the target office blood pressure.

Elderly patients ≥75 years of age often have organ damage; therefore, careful management with antihypertensive therapy is needed taking into consideration the QOL, using an intermediate target blood pressure of 150/90 mmHg. Patients with a high pulse pressure are expected to have advanced atherosclerosis, which means that a slow and careful reduction of blood pressure should be achieved.

### *Step 5C: DM Management Goals*

- *Targets for the index of glycemic control and management of blood glucose should be set for each patient taking into consideration their age and disease condition. Generally, the target should be an HbA1c (NGSP) level of < 7.0%.*

As a risk factor for CVD, DM occupies a very important position along with dyslipidemia and hypertension. Patients with persistent poor glycemic control, such as an HbA1c (NGSP) level of ≥8.4% or

organ damage (e.g., retinopathy, nephropathy or PAD) have an especially high risk (**Table 5**). The risk of developing CVD is much higher in women with DM than in women without DM.

To prevent the development/progression of CVD in patients with DM, obtaining good glycemic control alone is insufficient, and comprehensive and strict management of risk factors, such as dyslipidemia, hypertension, obesity (visceral fat accumulation) and smoking, is required.

***Step 5D: Management of Other Conditions***

• *Metabolic syndrome is based on the excessive accumulation of visceral fat and is characterized by a cluster of risk factors for CVD. Simultaneous with treating each risk factor, reducing obesity, particularly visceral fat, should be a management target.*

• *In order to address other diseases closely associated with lifestyle, such as hyperuricemia, an appropriate treatment/management goal to prevent CVD should be set for each patient.*

Epidemiological studies performed in Western countries and Japan have shown that the clustering of risk factors, such as that observed in metabolic syndrome, is associated with an increased risk of CVD. In addition to managing each risk factor, reducing visceral fat, the accumulation of which is the basis for metabolic syndrome (i.e., reducing obesity and waist circumference), should be considered. For such patients, active guidance on anti-obesity measures should be provided, with a 5% decrease in body weight or waist circumference after three to six months being the immediate target, and achievement of the target should be assessed over time.

The serum uric acid level is an independent predictor of future hypertension[4, 5] and is associated with the development and/or progression of CKD[6, 7]. An elevated uric acid level reflects an increased frequency of metabolic syndrome[8]. Therefore, even in cases of asymptomatic hyperuricemia without gout or renal calculi, therapeutic intervention should be considered if there is a history of hypertension, DM or CAD and the uric acid level is ≥8.0 mg/dL. Lifestyle modification is also the basis of treatment in such cases[9].

## 6. Therapy (Lifestyle Modification)

***Step 6: Lifestyle Modification***

• *Lifestyle modification is positioned as the foundation of prevention of CVD. All patients should be provided with adequate guidance regarding lifestyle modification.*

• *If a patient is monitored while only being encouraged to modify his/her lifestyle, he/she should desirably make regular hospital visits to maintain motivation and improve treatment adherence and effects.*

**Table 8.** Lifestyle Modification for the Prevention of CVD

1. Stop smoking and avoid passive smoking.
2. Refrain from overeating and maintain an ideal body weight.
3. Reduce intake of meat fat, dairy products and egg yolk and increase the intake of fish and soy products.
4. Increase intake of vegetables, fruit, unrefined grains and seaweed.
5. Reduce intake of food containing too much salt.
6. Avoid excessive alcohol consumption.
7. Perform aerobic exercise for at least 30 min daily.

Lifestyle modification forms the basis of the prevention of CVD, and introducing drug therapy without careful consideration should be avoided. After drug therapy is commenced, continued guidance on lifestyle modification should be provided (**Table 8**).

Smoking is one of the most important factors that can be targeted for intervention among the causes of CVD. In order to prevent CVD, smoking cessation should be recommended for people of all ages and both sexes. The increased risk of CAD observed in nonsmokers due to passive smoking is also a serious issue.

A BMI of ≥25 is considered to indicate obesity. For obese individuals, particularly patients with visceral fat accumulation (metabolic syndrome), a 5% decrease in body weight and/or waist circumference should be the immediate target.

Optimizing the total energy intake and nutrient balance and modifying inappropriate dietary habits and eating behaviors form the basis of treatment in patients with risk factors, such as dyslipidemia, hypertension, DM and obesity. Soluble dietary fiber should be consumed abundantly, while the intake of cholesterol and saturated fatty acids should be reduced. Patients with hypertension are recommended to limit their intake of salt to <6 g/day.

It has been demonstrated that exercise can improve dyslipidemia (e.g., increase the level of HDL-C) as well as exert hypotensive effects, improve insulin resistance and achieve hypoglycemic effects. Engaging in moderate aerobic exercise (approximately 50% of maximum oxygen uptake) for at least 30 minutes per day at least three times per week (daily if possible) or at least 180 minutes per week is desirable. For patients with hypertension, except those with mild to moderate blood pressure elevation (160 to 179/100 to 109 mmHg) and no CVD, prior medical examinations are needed. In DM patients with poor glycemic control

(e.g., positive urine ketones), retinopathy, CVD, renal failure, peripheral neuropathy or autonomic neuropathy, a specialist should be consulted regarding the appropriateness or need for restricting exercise therapy. In this context, a meta-analysis of patients with a history of CAD demonstrated that exercise therapy alone can improve the prognosis[10, 11].

**7. Therapy (Drug Therapy)**

Following the initiation of drug therapy, lifestyle modification (Step 6) should be continued.

***Step 7A: Drug Therapy for Dyslipidemia***

- *If a patient cannot achieve their target LDL-C level following adequate lifestyle modification in primary prevention, drug therapy should be considered according to the weight of the risk.*
- *If a patient in category I persistently has an LDL-C level of ≥ 180 mg/dL, drug therapy should be considered.*
- *Statins are recommended for the treatment of hyper-LDL cholesterolemia.*
- *In patients with high-risk hyper-LDL cholesterolemia, the use of ezetimibe in combination with a statin should be considered.*
- *In patients with high-risk hyper-LDL cholesterolemia, the use of eicosapentaenoic acid (EPA) in combination with a statin should be considered.*
- *In patients with hypertriglyceridemia accompanied by hypo-HDL cholesterolemia, drug therapy with fibrates, nicotinic acid derivatives or other similar drugs should be considered according to the weight of the risk.*

There is abundant evidence that LDL-C-lowering therapy with statins can prevent CVD. If a patient with dyslipidemia cannot achieve their target level with a single drug, dose escalation of the drug or the use of combination therapy should be considered. The Japan EPA Lipid Intervention Study (JELIS) conducted in Japanese patients with hyper-LDL cholesterolemia revealed that those who received statins in combination with EPA developed significantly fewer major coronary events compared with patients who received statins alone[12].

In patients with renal dysfunction, since rhabdomyolysis occurs more frequently with the use of statins or fibrates, the combination therapy of statins and fibrates is contraindicated.

***Step 7B: Drug Therapy for Hypertension***

- *In patients with hypertension, drug therapy should be considered if the office blood pressure is >140/90 mmHg (home blood pressure: 135/85 mmHg) after a certain period of adequate lifestyle modification (three months in low-risk patients or one month in moderate-risk patients). In high-risk patients with hypertension complicated by DM, CKD, CVD or organ damage, the initiation of drug therapy should be considered while the patient is encouraged to modify their lifestyle (**Table 4**).*
- *One of the following five types of drugs should be selected as the first choice: Ca-antagonists, angiotensin II-receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACE inhibitors), diuretics or β-blockers (including α/β-blockers). The drug should be selected according to each patient's condition while giving due consideration to the positive indications, relative contraindications and contraindications of each drug.*
- *Combination therapy is required in many cases to achieve the goal of hypertension treatment. Recommended combination therapies include renin-angiotensin (RA) inhibitors (ARBs or ACE inhibitors) and Ca-antagonists, RA inhibitors and diuretics, Ca-antagonists and diuretics and Ca-antagonists and β-blockers.*

When antihypertensive treatment is administered, appropriate drugs should be selected according to each patient's condition.

RA inhibitors are recommended as the first-line drugs for patients with organ damage, such as those with proteinuria or renal dysfunction, heart failure, old myocardial infarctions (MIs) or DM. However, for patients with renal dysfunction (a serum creatinine level of >2.0 mg/dL), careful administration is desirable since the renal function may be worsened. The administration of such drugs in pregnant or lactating women is contraindicated. When RA inhibitors are used in combination with K-sparing diuretics, attention should be paid to the possibility of hyperkalemia. In patients with bilateral renovascular hypertension, caution should be exercised since rapid progression of renal dysfunction may be observed following the administration of RA inhibitors.

β-blockers are not used as first-line drugs in the elderly or patients with impaired glucose tolerance, since monotherapy or combination therapy with diuretics may exacerbate glucose/lipid metabolism. Generally, the combination of β-blockers and diuretics is not recommended.

***Step 7C: Drug Therapy for DM***

- *In patients with non-insulin-dependent type 2 DM, drug therapy should be considered if good glycemic control cannot be achieved after two to three months of lifestyle modification, including adequate diet and exercise therapy.*
- *However, drug therapy may be administered in the early stage of the disease in patients exhibiting a poor*

*response to lifestyle modification or a certain degree of metabolic disorder.*
*• Available oral drugs include sulfonylureas (SUs), fast-acting insulin secretagogues, α-glucosidase inhibitors, biguanides, thiazolidines and dipeptidyl peptidase-4 (DPP-4) inhibitors.*
*• Glucagon-like peptide (GLP)-1-receptor agonists are available in injectable forms.*
*• Insulin therapy should preferably be initiated after consulting diabetes specialists. Insulin may be administered in combination with oral drugs.*

When drug therapy is prescribed, attention should always be paid to hypoglycemia, and the patient should be provided adequate guidance.

For insulin-dependent diabetic patients, such as those with type 1 DM, insulin therapy is required; therefore, referral to and close cooperation with a specialist during ongoing treatment is needed. For patients with type 2 DM who have severe metabolic derangement, severe infection or a history of invasive surgery, insulin therapy is required, and a specialist should be consulted.

For other non-insulin-dependent patients, adequate education should be provided regarding lifestyle modification, such as appropriate diet and exercise therapy. If the target for glycemic control cannot be achieved after two to three months of treatment, the initiation of drug therapy should be considered. The glycemic control target will vary for each patient according to the patient's condition.

In patients with increased insulin resistance, such as those with obesity, biguanides and thiazolidines are good choices. For patients with a decreased insulin secretory capacity, SUs and DPP-4 inhibitors are indicated. To correct postprandial hyperglycemia, fast-acting insulin secretagogues, α-glucosidase inhibitors and DPP-4 inhibitors are good options.

GLP-1-receptor agonists are analogs of the incretin GLP-1 that promote insulin secretion and decrease both fasting blood glucose and postprandial blood glucose.

Attention should be paid to adverse reactions specific to each drug, including weight gain with SUs, gastrointestinal symptoms (such as abdominal bloating and diarrhea) with α-glucosidase inhibitors, reactions to the use of iodinated contrast agents with biguanides and heart failure or edema caused by thiazolidines.

Various insulin preparations with different durations of action and dosage forms are available. Insulin therapy should be initiated after consulting with a diabetes specialist, if possible. Selecting the proper insulin preparation and adjusting the timing and number of injections according to the patient's lifestyle is required. Providing guidance regarding the procedures of injection and self-monitoring of blood glucose is also important.

### *Step 7D: Other Drug Therapy*

*• Antiplatelet therapy is effective for the secondary prevention of CAD and noncardiogenic cerebral infarction. Attention should always be paid to the development of adverse drug reactions, such as hemorrhagic complications, during the administration of this regimen.*

The inhibitory effects of low-dose aspirin (75 to 150 mg/day) on cardiovascular events in patients with a history of MI were demonstrated in a meta-analysis[13], and the effectiveness of this medication in Japanese individuals was shown in the Japanese Antiplatelet Myocardial Infarction Study (JAMIS)[14, 15].

However, the results of a recent meta-analysis revealed that the inhibitory effects of aspirin treatment on cardiovascular death in the primary prevention of CVD may be offset by an increased risk of hemorrhagic complications[16], suggesting that administration without careful consideration should be avoided[17]. Among Japanese individuals, the J-PAD showed no inhibitory effects of low-dose aspirin on cardiovascular events in patients with type 2 DM[18], while a subanalysis demonstrated inhibition of cardiovascular events in elderly subjects ≥65 years of age and patients with moderate renal dysfunction[18].

To prevent the recurrence of noncardiogenic cerebral infarctions, such as atherothrombotic and lacunar infarctions, the administration of low-dose aspirin or clopidogrel is recommended. Cilostazol is also effective for decreasing the recurrence of cerebrovascular disease. However, managing blood pressure is the most important measure for preventing the recurrence of lacunar infarctions[19].

Cilostazol is effective to some extent in improving the symptoms of PAD, such as intermittent claudication. The administration of aspirin is effective for improving patency following revascularization or endovascular treatment. Furthermore, aspirin and clopidogrel have been demonstrated to be effective for preventing cerebrovascular death in patients with PAD[20, 21].

## Footnotes

This is an English version of the guidelines of the Japan Atherosclerosis Society (chapter 2) published in Japanese in June 2012.

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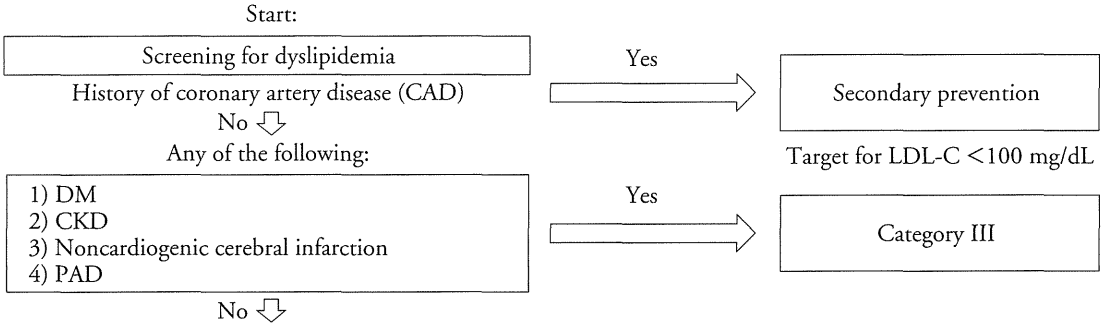
**Supplementary Table 1.** Relative Risk Charts for Patients with a Low Absolute Risk (based on the risk charts of the NIPPON DATA80)

| | | | | Nonsmokers | | |
|---|---|---|---|---|---|---|
| Systolic blood pressure | | | | | | |
| Second-degree or higher hypertension (≥160 mmHg) | 2.2 | 2.8 | 3.6 | 4.6 | 5.8 | 7.4 |
| First-degree hypertension (140-159 mmHg) | 1.7 | 2.2 | 2.8 | 3.5 | 4.5 | 5.7 |
| Normal (≤140) | 1.0* | 1.3 | 1.6 | 2.1 | 2.6 | 3.4 |
| TC category (mg/dL) | 160-179 | 180-199 | 200-219 | 220-239 | 240-259 | 260+ |
| | | | | Smokers | | |
| Systolic blood pressure | | | | | | |
| Second-degree or higher hypertension (≥160 mmHg) | 3.2 | 4.1 | 5.2 | 6.6 | 8.4 | 10.7 |
| First-degree hypertension (140-159 mmHg) | 2.5 | 3.1 | 4.0 | 5.1 | 6.5 | 8.2 |
| Normal (≤140 mmHg) | 1.4 | 1.8 | 2.3 | 3.0 | 3.8 | 4.8 |
| TC category (mg/dL) | 160-179 | 180-199 | 200-219 | 220-239 | 240-259 | 260+ |

*Reference group

To calculate the relative risks used in this table, the representative values in each risk factor category were used. The representative values in each TC category were set at 160, 190, 210, 230, 250 and 270, the representative values in each systolic blood pressure category were set at 110 (normal), 150 (degree I) and 170 (degree II) and the patients were assumed to not have DM. The relative risk for patients who are nonsmokers with a TC level of 160 to 179 and a normal blood pressure was used as the reference value (i.e., relative risk: 1.0). For the sake of convenience, the relative risks were calculated assuming that the patients were men 40 years of age because the values cannot be calculated if the sex and age are not fixed. If the TC level cannot be used, the LDL-C + 80 value should be used.

**Supplementary Table 2.** Simple Chart Based on Sex, Age and the Number of Risk Factors for Predicting the Absolute Risk of CAD




| Sex | Age | Determined based on the number of risk factors: (1) Hypertension (2) Smoking (3) Hypo-HDL cholesterolemia (HDL-C <40 mg/dL) (4) Family history of premature CAD (first-degree male relatives aged <55 years or female relatives aged <65 years) (5) Impaired glucose tolerance (excluding DM) | Absolute risk of CAD (%) | Category* |
|---|---|---|---|---|
| Men | 40-49 years (Also applied to persons aged 30-39 years) | 0 | 0.23 | Category I |
| | | 1-2 | 0.32-0.55 | Category II |
| | | ≥3 | 0.48-0.83 | Category III |
| | 50-59 years | 0 | 0.63 | Category II |
| | | 1 | 0.91-1.08 | Category II |
| | | ≥2 | 1.55 | Category III |
| | 60-69 years (Also applied to persons aged ≤74 years) | 0 | 1.78 | Category II |
| | | ≥1 | 2.55-4.31 | Category III |
| Women | 40-59 years | 0-1 | 0.10-0.20 | Category I |
| | | ≥2 | 0.24 | Category II |
| | 60-69 years (Also applied to persons aged ≤74 years) | 0-1 | 0.87-1.83 | |
| | | ≥2 | 2.19 | Category III |

In this simple chart, the serum level of LDL-C was set at 170 (TC=250), which exceeded the upper limit of the least strict management target (LDL-C=160). Then, the absolute risk of CAD death was calculated using the NIPPON DATA risk chart as follows:

1) For age, the median (men: 45, 55 and 65 years; women: 50 and 65 years) was used.
2) The number of risk factors was calculated according to the presence or absence of hypertension (presence: SBP=160; absence: SBP=120) and the presence or absence of smoking, of which the maximum number was 2.
3) In cases in which the number of risk factors was ≥3, the absolute risk was estimated based on the assumption that the third risk factor (other than hypertension and smoking) increases the risk 1.5-fold.

*Depending on the level of additional risk factors, the absolute risk may not always be within the same range as in Fig. 1. Furthermore, because the relative risk for patients in the same sex and age group is also taken into consideration, it should be noted that the category may not always be consistent with the range of the estimated absolute risk determined using the NIPPON DATA risk charts. This chart may be used as a convenient method if the NIPPON DATA risk chart is not readily available.

Management Category Target for the LDL-C level:

Category I <160 mg/dL, Category II <140 mg/dL, Category III <120 mg/dL, Secondary prevention <100 mg/dL

Committee Report 3

# Diagnostic Criteria for Dyslipidemia

## Executive Summary of the Japan Atherosclerosis Society (JAS) Guidelines for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases in Japan — 2012 Version

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Committee for Epidemiology and Clinical Management of Atherosclerosis

Epidemiological studies conducted in Japan as well as Western countries have shown that higher levels of LDL-cholesterol (LDL-C)[1], total cholesterol (TC)[2-7], non HDL-cholesterol (non HDL-C)[8], and triglyceride (TG)[9, 10] and lower levels of HDL-C[5, 11-13] are associated with a higher risk of coronary artery disease (CAD) (**Fig. 1**). At present, the absolute risk (incidence and mortality) of CAD in Japan is much lower than that observed in Western countries[14-17]; however, due to recent increases in the LDL-C and TC levels in Japanese individuals as a result of Westernization of the Japanese lifestyle[18, 19], and the findings of a report showing that the incidence of CAD is increasing in some regions of Japan[19, 20], there is concern that the incidence of CAD will rise throughout Japan. Therefore, these guidelines define diagnostic criteria for assessing dyslipidemia during screening to prevent the development of arteriosclerosis from the perspective of preventing CAD, as shown in **Table 1**.

According to the diagnostic procedures, first, the TC, TG and HDL-C levels are measured in the morning after overnight fasting to calculate the LDL-C level using the Friedewald formula (LDL-C = TC − HDL-C − TG/5). This formula cannot be used in a non-fasting state or when the TG level is ≥ 400 mg/dL because large errors in the LDL-C level may occur. Although direct measurement methods for determining the LDL-C level have been applied clinically, significant problems have been found concerning variations in accuracy and the results obtained between kits, especially in cases of high TG levels[21]. Therefore, using the non HDL-C level is recommended when the TG level is ≥ 400 mg/dL. The non HDL-C level is calculated by subtracting the HDL-C level from the TC level.

Lipid standardization in clinical laboratories in Japan has been judged internationally to be very accurate for the TC levels and fairly accurate for the HDL-C levels[22]. Nevertheless, the accuracy of TG and LDL-C measurements remains inadequate[22, 23]; thus, further standardization is warranted.

### 1. Hyper-LDL Cholesterolemia

The Framingham study and many other epidemiological studies conducted in Western countries have shown that the incidence and mortality of CAD increase in association with increases in the levels of TC and LDL-C. In addition, in Japan, epidemiological studies, such as the NIPPON DATA80[2], Suita[24], JALS[25], CIRCS[1], Hiroshima/Nagasaki[7], MHW Primary Hyperlipidemia[26], Okinawa cohort[27] and Ehime epidemiological[10] studies and epidemiological studies conducted in 76 workplaces in Japan (the 3M Study)[4], have confirmed that the relative risk of CAD increases continuously in association with increases in the levels of LDL-C and TC.

The NIPPON DATA80, a prospective epidemiological study conducted in Japan, demonstrated that the relative risk of CAD-related death in individuals with a TC level of 200-219 mg/dL, 220-239 mg/dL, 240-259 mg/dL and ≥ 260 mg/dL is 1.4-, 1.6-, 1.8- and 3.8-fold higher, respectively, than that observed in individuals with a TC level of 160-179 mg/dL (**Fig. 1a**)[2]. In men, in particular, mortality from CAD increases continuously in association with increases in the TC (LDL-C) levels, with no distinct threshold.

Meanwhile, studies conducted in Western countries regarding interventions for hypercholesterolemia, including lifestyle modification, have revealed that

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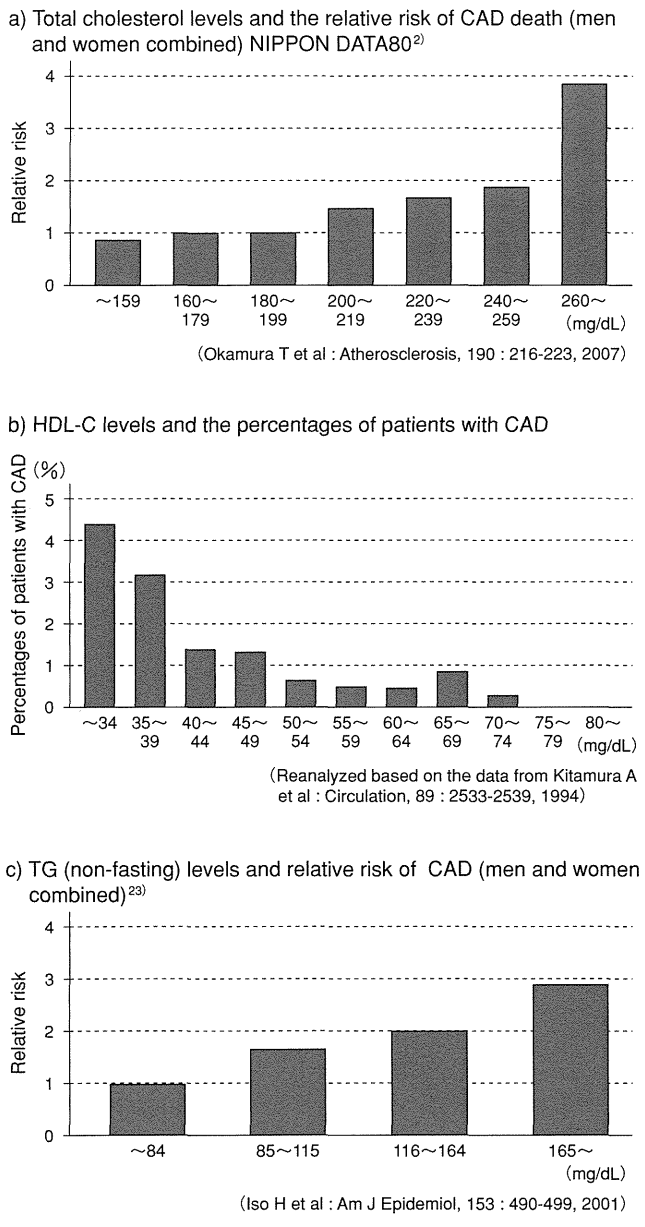


**Fig. 1.** Serum Lipids and Risk for CAD

intervention significantly decreases the incidence of CAD. In addition, in Japan, the results of large-scale clinical studies have recently shown that treatment for hyper-LDL cholesterolemia is clinically beneficial for Japanese individuals[28-31].

In the U.S. guidelines NCEP-ATP Ⅲ, based on the relationship between the TC levels and CAD mortality reported in the MRFIT[32], the cutoff value for hypercholesterolemia is a TC level of 240 mg/dL, the level at which the relative risk is 2-fold higher than that observed at a TC level of 200 mg/dL[33]. As described above, the absolute risk of CAD in Japanese individuals is much lower than that observed in Westerners. In order to maintain this low risk, the use of early prevention measures is needed.

Based on the above findings, a TC level of 220 mg/dL, the level at which the relative risk shown in the NIPPON DATA80 is approximately 1.5-fold higher than that observed at a TC level of <180 mg/dL, was used as the cutoff value for screening Japanese individuals in terms of the prevention and treatment of CAD, and the corresponding LDL-C level of 140 mg/dL was defined as the cutoff value for the diagnosis of hyper-LDL cholesterolemia.

The CIRCS, an epidemiological study recently conducted in Japan[1], showed that the incidence of CAD in subjects with an LDL-C level of 80 to 99 mg/dL, 100 to 119 mg/dL, 120 to 139 mg/dL and ≥140 mg/dL is 1.4-, 1.7-, 2.2- and 2.8-fold higher, respectively, than that observed in subjects with a LDL-C level of <80 mg/dL. In the presence of multiple risk factors, the incidence of and mortality from CAD also increase in Japanese individuals. Since the incidence of and mortality from CAD in patients with multiple risk factors were found to be higher than those observed in patients without such factors, even at the same LDL-C levels, and patients with diabetes mellitus (DM) developed CAD at lower LDL-C levels of approximately 30 to 40 mg/dL as frequently as patients without DM in a subanalysis of primary prevention in the J-LIT study[34], it has been suggested that the degree of the increased risk of CAD associated with the LDL-C level changes depending on comorbidities. As a result of these concerns, these guidelines define an LDL-C level of 120-139 mg/dL as the borderline level at which the effects of other risk factors should be carefully considered when screening Japanese individuals for dyslipidemia.

## 2. Hypo-HDL Cholesterolemia

Having a low level of HDL-C places a patient at risk for developing CAD. Conversely, a higher HDL-C level is associated with a decreased risk of CAD (**Fig. 1b**)[11, 35]. In the NIPPON DATA90, the HDL-C level was found to be significantly inversely correlated with overall mortality and stroke mortality during the 9.6-year observation period[36]. Community and worksite-based cohort studies have shown that an HDL-C level <40 mg/dL is associated with an increased risk of CAD[11, 37-39]. In the J-LIT, a cohort study, simvastatin-treated primary prevention patients[12] and secondary prevention patients[13] with an HDL-C level <40 mg/dL were found to have a

**Table 1.** Dyslipidemia: Diagnostic Criteria for Screening (Fasting*)

| | | |
|---|---|---|
| Low-density lipoprotein cholesterol (LDL-C) | ≥140 mg/dL | Hyper-LDL cholesterolemia |
| | 120-139 mg/dL | Borderline hyper-LDL cholesterolemia** |
| HDL-C | <40 mg/dL | Hypo-HDL cholesterolemia |
| Triglycerides (TGs) | ≥150 mg/dL | Hypertriglyceridemia |

• The LDL-C level is calculated using the Friedewald formula (TC − HDL-C − TG/5) (if TG <400 mg/dL).
• If the TG level is ≥ 400 mg/dL or non-fasting blood is used, the non HDL-C (TC − HDL-C) level should be used. The cutoff value is LDL-C +30 mg/dL.
*A "fasting state" is defined as having fasted for ≥ 10 to 12 hours. The consumption of liquids with no calories, such as water and tea, is permitted.
**If borderline hyper-LDL cholesterolemia is diagnosed during screening, the presence of high-risk conditions should be assessed and the need for treatment should be considered.

1.3- and 1.6- fold higher relative risk of CAD, respectively, than those with an HDL-C level of 40-49 mg/dL. Based on these findings, these guidelines define an HDL-C level of <40 mg/dL as the cutoff value for screening for hypo-HDL cholesterolemia. In general, women exhibit higher HDL-C levels than men[36, 39, 40]; however, there is currently insufficient evidence to support the existence of a relationship between sex differences in the HDL-C levels and the incidence of CAD. Therefore, these guidelines used the same cutoff value for both women and men.

### 3. Hypertriglyceridemia

Many reports have shown that a high TG level is associated with a risk of developing CAD in Asia, Oceania[41] and Japan[9, 10, 39, 42, 43] as well as in Western countries[44]. In some of these studies, the TG level was found to be associated with the risk of CAD even when the HDL-C level was corrected[9, 41, 42, 44]. In the U.S., hypertriglyceridemia is defined as a fasting TG level of ≥150 mg/dL based on the Framingham study[45]. Traditionally, the TG level has been measured using fasting blood; however, one report indicates that the non-fasting TG level more accurately predicts cardiovascular events[46]. Epidemiological studies conducted in Japan have shown that the incidence of CAD increases when the fasting TG is ≥150 mg/dL[10, 39, 43] and that the incidences of myocardial infarction, exercise-induced angina and sudden death increase when the non-fasting TG level is ≥165 mg/dL (**Fig. 1c**)[9]. Moreover, many reports have also shown that hypertriglyceridemia is a risk factor for cerebral infarction, although this association is weaker than that observed for CAD[39, 41, 47-49]. Considering these findings, these guidelines define a TG level of ≥150 mg/dL as the cutoff value for screening for hypertriglyceridemia; however, hypertriglyceridemia often reflects other pathological conditions, such as increased levels of remnant lipoproteins or small, dense LDL, complications of hypo-HDL cholesterolemia and the presence of metabolic syndrome. Therefore, other conditions associated with increased TG levels should be carefully assessed.

### 4. Non HDL Cholesterol

If hypertriglyceridemia exists, especially when the TG level is ≥400 mg/dL, the correct LDL-C level cannot be calculated because the Friedewald formula is not applicable and the direct measurement method is problematic. In such cases, the non HDL-C level is a useful and simple index calculated by subtracting the HDL-C level from the TC level. Some investigators consider the non HDL-C level to be superior to the LDL-C level in terms of predicting the development of atherosclerotic diseases because the non HDL-C level incorporates all atherogenic lipoproteins, including remnant lipoproteins[50, 51]. Recently, many epidemiological studies have examined the relationship between the non HDL-C level and the risk of CAD in Japan[8, 24, 25, 49, 52]. The non HDL-C level exhibits the same relationship with the incidence of myocardial infarction as the LDL-C level, with both parameters demonstrating comparable ability to predict the development of myocardial infarction[24]. On the other hand, one study showed that the non HDL-C level is superior to the TC level in terms of predicting the incidence of myocardial infarction[25]. The incidence and mortality of CAD and myocardial infarction markedly increase in men with a non HDL-C level of ≥170-180 mg/dL, while no specific tendencies have been observed in women[8, 24, 25, 52]. One study investigated the risk of myocardial infarction associated with the non HDL-C level in the presence or absence of hypertriglyceridemia[49]. In that report, the risk of myocardial infarction markedly increased in the group with both hypertriglyceridemia (TG ≥150 mg/dL) and a

non HDL-C level of ≥ 190 mg/dL. In a subanalysis of the JELIS that compared the groups that achieved both LDL-C and non HDL-C management goals, the other groups exhibited higher incidences of CAD[53]. Recently, it was demonstrated that the non HDL-C level in Japanese individuals is equal to LDL-C +30 mg/dL, the same as that observed in the U.S[54, 55]. Based on these findings, these guidelines defined a non HDL-C level of ≥ 170 mg/dL as the cutoff value for screening.

## Footnotes

This is an English version of the guideline from the Japan Atherosclerosis Society (chapter 3) published in Japanese in June, 2012.

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# Novel Collagen/Gelatin Scaffold with Sustained Release of Basic Fibroblast Growth Factor: Clinical Trial for Chronic Skin Ulcers

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Chronic skin ulcers such as diabetic ulcers and venous leg ulcers are increasing and are a costly problem in healthcare. We have developed a novel artificial dermis, collagen/gelatin sponge (CGS), which is capable of sustained release of basic fibroblast growth factor (bFGF) for more than 10 days. The objective of this study was to investigate the safety and efficacy of CGS impregnated with bFGF in the treatment of chronic skin ulcers. Patients with chronic skin ulcers that had not healed in at least 4 weeks were treated with CGS impregnated with bFGF at 7 or 14 $\mu g/cm^2$ after debridement, and the wound bed improvement was assessed 14 days after application. Wound bed improvement was defined as a granulated and epithelialized area on day 14 with a proportion to the baseline wound area after debridement of 50% or higher. The wound area, the wound area on day 14, and the granulation area on day 14 were independently measured by blinded reviewers in a central review using digital images of wounds taken with a calibrator. Patients were followed up until 28 days after application to observe the adverse reactions related to the application of CGS. From May 2010 to June 2011, 17 patients were enrolled and, in 16 patients, the wound bed improved. Among the randomized patients in step 2, no significant difference was seen between the low-dose group and the high-dose group. No serious adverse reactions were observed. Adverse reactions with a clear causal relationship to the study treatment were mild and patients quickly recovered from them. This study is the first-in-man clinical trial of CGS and showed the safety and efficacy of CGS impregnated with bFGF in the treatment of chronic skin ulcers. This combination therapy could be a promising therapy for chronic skin ulcers.

## Introduction

Chronic skin ulcers caused by diabetes mellitus, venous insufficiency, pressure sores, collagen disease, trauma, or radiation are an increasing and costly problem in healthcare.[1–4] Diabetic foot ulcer is one of the most common complications in diabetic patients and is a leading cause of major amputations of the lower limbs.[1,3,4] Venous ulcers are usually recurrent and associated with impaired quality of life.[5] With the development of tissue engineering and cell culture techniques, bioengineered skin substitutes containing fibroblasts or keratinocytes have been used clinically and reported to be effective for the treatment of chronic ulcers.[6–8] Recently, preclinical and clinical studies have shown that mesenchymal stem cells such as bone marrow-derived or adipose tissue-derived stem cells may be effective in wound healing.[9,10] These cell therapies are promising treatments; however, their high cost and the limited supply of skin substitutes containing living cells are significant issues for further clinical usage.[10]

In addition to these cell therapies, genetically derived growth factors have been developed in association with advances in molecular biology. Some growth factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and epidermal growth factor have already been clinically used for the treatment of chronic skin ulcers.[11–13] bFGF, which was identified in 1974, promotes the proliferation of fibroblasts and capillary formation and

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accelerates tissue regeneration.[14,15] In Japan, human recombinant bFGF (FIBRAST SPRAY; Kaken Pharmaceutical, Tokyo, Japan) has been clinically used for chronic skin ulcers since 2001, and its clinical effectiveness has been demonstrated.[14,16] Recently, combination therapy involving bFGF and artificial dermis has been reported to accelerate dermis-like tissue formation, not in the treatment of acute and chronic skin ulcers alone.[17–19] A novel artificial dermis, collagen/gelatin sponge (CGS) containing a 10 wt% concentration of acidic gelatin, was developed in a previous study.[20] CGS can sustain the release of positively charged growth factors such as bFGF for more than 10 days.[20] In preclinical studies using mice and beagle dogs, CGS itself could be used as a scaffold for dermal regeneration, the same as conventional artificial dermis, and CGSs impregnated with 7 or 14 $\mu g/cm^2$ bFGF accelerated neovascularization and the formation of dermis-like tissue two or three times earlier than with conventional artificial dermis.[21–23]

In view of the above, combination therapy with CGS and bFGF is expected to have an effect comparable to tissue engineering products containing cells on promoting wound healing, even in patients with chronic skin ulcers. Thus, an exploratory clinical study was designed to investigate the safety and efficacy of this combination therapy in the treatment of chronic skin ulcers. In chronic ulcers caused by diabetes mellitus, venous insufficiency, pressure sores, or collagen disease, granulation tissue is not easily formed. In this study, the null hypothesis that the proportion of patients with wound bed improvement was 10% or less within 14 days was tested.

## Materials and Methods

### *Ethical considerations*

This study was conducted in compliance with Good Clinical Practice and in agreement with the latest revision of the Declaration of Helsinki, Pharmaceutical Affairs Law, and all applicable Japanese laws and regulations, as well as any local laws and regulations and all applicable guidelines. This protocol and any amendments have Institutional Review Board approval from Kyoto University Hospital.

### *Study design and patients*

This study was an open-label, randomized, multiple dose, controlled clinical trial to evaluate the safety and efficacy of CGS impregnated with bFGF in the treatment of chronic skin ulcers that were not expected to heal with conventional treatments. It was conducted at Kyoto University Hospital.[24]

The design was previously reported in detail.[24] Two groups, low-dose (7 $\mu g/cm^2$ bFGF impregnation) and high-dose (14 $\mu g/cm^2$ bFGF impregnation) groups, were arranged. In the initial step (step 1), three patients were enrolled in the low-dose group and, after confirming the safety of this step, fourteen patients were randomized to the low-dose or high-dose bFGF group in step 2 at a ratio of 1:1 without stratification using a computer-generated random sequence (Fig. 1). Data management, randomization, and statistical analysis were managed by the independent data center in the Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital.

The main inclusion criteria were patients aged 20 years or older, who had given informed consent, with the presence of chronic skin ulcers as follows: not healing for at least 4 weeks with conventional treatments; skin graft was not expected to take place; and could be completely covered by a $70 \times 100$ mm CGS. If chronic skin ulcers were present on the lower extremities, the skin perfusion pressure had to be $\geq 30$ mmHg at a site proximal or distal to those ulcers. The exclusion criteria were as follows: uncontrolled diabetes mellitus; requiring continued use of oral corticosteroid therapy; having a history of malignant tumor; or having a history of allergy to porcine-derived products. The details were described in our previous article.[24] If a patient had multiple intractable skin ulcers, CGSs were applied to all these ulcers for study therapy. The largest eligible ulcer was selected and evaluated for efficacy and safety, and the others were evaluated only for safety.

### *Preparations of CGS impregnated with bFGF*

The CGS was a modified version of the conventional bilayered artificial dermis (Pelnac®; Gunze Co., Ltd., Kyoto, Japan) and consisted of an upper silicone sheet (0.12 mm in thickness) and a lower sponge (3 mm in thickness).[20,25,26] In this study, the size of the CGS was $82 \times 120$ mm. The investigator or sub-investigator prepared CGS impregnated with bFGF at 7 $\mu g/cm^2$ (low dose) or 14 $\mu g/cm^2$ (high dose) in the operating room just before application (Fig. 2).

### *The combination therapy with CGS and bFGF*

In this study, human recombinant bFGF (FIBRAST SPRAY; Kaken Pharmaceutical) was used. In the impregnation of the low-dose group, 1000 μg of bFGF was dissolved in 14.4 mL of distilled water and 10 mL of this solution was applied to CGS. In the impregnation of the high-dose group, 2000 μg of bFGF was dissolved. About 10 min were needed for impregnation.

After surgical debridement by a single operator, CGS impregnated with bFGF at 7 or 14 $\mu g/cm^2$ and cut according to the shape of the wound was applied and sutured to the surrounding skin. Reapplication of the device within 3 days of initial application was allowed if necessary because of hematoma formation or dislocation of the device.

After the application of CGS, dressings were changed as necessary. Patients were hospitalized until day 7 to ensure stabilization of the applied CGS and for the safety assessment of study participants. On day 14 after application, the sutures and silicone sheet of CGS were removed.

The additional use of bFGF or a collagen-based artificial skin was prohibited until day 28. After day 29, no particular restrictions were imposed.

### *Evaluation of treatment and endpoints*

Using a digital camera (Canon EOS Kiss Digital X; Canon, Inc., Tokyo, Japan), digital images of the wounds were taken with a calibrator (CASMATCH®; BEAR Medic Corp., Tokyo, Japan) placed on the skin adjacent to the wound. The color and size of images were adjusted using CASMATCH and image editing software (Adobe Photoshop; Adobe Systems) to assess the wound and granulation areas under a standardized procedure. As with the primary endpoint, the baseline wound area, the wound area on day 14, and the granulation area on day 14 were independently measured by blinded reviewers in a central review.

The primary endpoint was wound bed improvement. Granulation tissue is wound connective tissue, which forms at the beginning of wound healing.[27,28] This highly fibrous tissue is usually pink because of the invasion of numerous small capillaries to supply oxygen and nutrients. The appearance of granulation tissue is a good sign of healing because, when a wound starts granulating, it means that the healing process of the wound has started.[27–29] The area of granulation tissue was measured as the granulation formation area in this study. An unhealed area was defined as an area with no epithelialization and no granulation formation. In this study, the percentage of wound bed improvement was defined as the value (%) calculated from the sum of the granulated and epithelialized areas on day 14 divided by the baseline wound area after debridement on day 0 multiplied by 100, and the patient was diagnosed with wound bed improvement if the wound bed improvement indicator was 50% or higher.[24] The use of 50% or more as the cutoff for the wound bed improvement indicator refers to the definition of the pressure ulcer healing assessment scale by the Japanese Society of Pressure Ulcers[30,31] because 50% or more granulation formation indicates the beginning of the wound healing process of retarded chronic ulcers and pressure ulcers.

The secondary endpoints were adverse reactions and serious adverse reactions, percentage of wound bed improvement, percentage of wound area reduction (defined as the value (%) calculated from the wound area of the ulcer on day 14 divided by the baseline wound area after debridement on day 0 multiplied by 100), and percentage of granulation area (defined as the value [%] calculated from the granulation area divided by the wound area on day 14 multiplied by 100). Adverse reactions and serious adverse reactions were documented according to the Medical Dictionary for Regulatory Activities (MedDRA), version 14.0.

### *Sample size*

This study was conducted to determine whether CGS impregnated with bFGF is promising for the treatment of chronic skin ulcers, as evaluated by wound bed improvement as the primary endpoint. The primary analysis was conducted using all data treated with CGS in step 1 and step 2. Since debridement and conventional therapies rarely lead to wound bed improvement in this patient population, the null hypothesis tested in this study was that the proportion of patients with wound bed improvement was 10% or less. This null hypothesis was also supported by previous trials.[32–34] Considering the minimum clinically important difference, the expected proportion of patients with wound bed improvement in this study was set to 50% or more. For exact testing based on a one-sample binomial distribution conducted with a one-sided significance level of 2.5% and a statistical power of 90% or higher, the required number of subjects was 14. Allowing for a drop-out rate of 20%, the total number of patients for registration was 17.

### *Statistical analysis*

Patients who had been registered for the study and had undergone investigational device application at least once were included in all statistical analyses. These patients became the statistical unit of analysis. The primary analysis was conducted using an exact test based on binomial distribution with a null proportion of 10% and a one-sided significance level of 2.5%. The 95% confidence interval of the proportion of patients with wound bed improvement was calculated using an exact method based on binomial distribution. The frequency/incidence of adverse reactions and serious adverse reactions that could be causally related to the investigational device was summarized by reaction and severity.

## Results

From May 2010 to June 2011, 17 patients (10 patients in the low-dose bFGF group and 7 patients in the high-dose bFGF group) were enrolled in this study. In step 2, 14 patients were randomized to the two dose groups. All 17 patients were included in the FAS and safety analysis set. In patients with multiple chronic skin ulcers, the largest ulcer ($n=17$) was analyzed for safety and efficacy and the other ulcers ($n=8$) were used only for safety. Baseline characteristics are shown in Table 1. All patients underwent the application of CGS impregnated with bFGF. One lesion was located on the right

Table 1. Study Patient Population

| | *Low-dose bFGF* (n = *10*) | *High-dose bFGF* (n = *7*) | *Total population* (n = *17*) |
|---|---|---|---|
| Age, mean (SD) | 54.7 (17.6) | 70.3 (7.6) | 61.1 (16.1) |
| Sex, male:female | 8:2 | 1:6 | 9:8 |
| Target ulcer area (SD) ($mm^2$) | 828 (1099) | 1523 (1312) | 1114 (1204) |
| Cause of ulcer (multiple selection) | | | |
| Venous leg ulcer | 0 | 4 | 4 |
| Arterial ulcer | 1 | 1 | 2 |
| Diabetic ulcer | 6 | 0 | 6 |
| Ulcers in association with collagen diseases | 2 | 1 | 3 |
| Traumatic ulcer | 2 | 0 | 2 |
| Decubitus ulcer | 0 | 1 | 1 |
| Cold burn | 1 | 0 | 1 |
| Chronic ulcer in scar tissue | 1 | 0 | 1 |
| Osteomyelitis or cellulitis | 0 | 1 | 1 |
| Static ulcer | 1 | 0 | 1 |

bFGF, basic fibroblast growth factor.

TABLE 2A. OUTCOMES IN TOTAL POPULATION

| | *Total population (n=17)* | p-*Value against the null hypothesis* |
|---|---|---|
| Wound bed improvement | 94.1% (95% CI: 71.3%–99.9%) | <0.001 |
| Percentage of wound bed improvement | 86.3% (95% CI: 71.2%–100%) | |
| Percentage of wound reduction | 87.3% (95% CI: 76.5%–98.0%) | |
| Percentage of granulation area | 86.3% (95% CI: 73.7%–98.9%) | |

TABLE 2B. SUBGROUP EFFECTS BETWEEN THE LOW-DOSE GROUP ($N$=7) AND THE HIGH-DOSE GROUP ($N$=7) IN STEP 2

| | *Low-dose bFGF (n=7)* | *High-dose bFGF (n=7)* | p-*Value between two groups in step 2* |
|---|---|---|---|
| Wound bed improvement | 100% (95% CI: 59.0%–100%) | 100% (95% CI: 59.0%–100%) | 1.00 |
| Percentage of wound bed improvement | 97.4% (95% CI: 94.0%–100%) | 88.1% (95% CI: 78.8%–97.5%) | 0.04 |
| Percentage of wound reduction | 77.5% (95% CI: 60.2%–94.8%) | 88.2% (95% CI: 69.2%–107.2% | 0.33 |
| Percentage of granulation area | 96.6%, (95% CI: 92.0%–100%) | 85.4%, (95% CI: 73.6%–97.3%) | 0.52 |

middle finger and the other lesions on the lower extremities. One patient enrolled in step 1 discontinued the study 4 days after reapplication because of infection, but the others completed the study.

Regarding the primary endpoint, wound bed improvement was achieved in 16 patients among the 17 (Table 2A). The proportion of patients with wound bed improvement within the total treatment population was 94.1% (95% CI: 71.3%–99.9%), which was significantly superior to the null hypothesis of 10% ($p<0.001$) (Table 2A). Significant subgroup effects were not found for diabetes, collagen disease, and trauma (results not shown). The outcomes in step 2 and for the total population are shown in Table 2B. We compared subgroup effects between the low-dose group ($n=7$) and the high-dose group ($n=7$) according to the randomized comparison in step 2. Among the randomized patients in step 2, significant difference was not seen between the low-dose group and the high-dose group ($p=1.00$). As for the secondary endpoint of efficacy, the percentage of wound bed improvement in the total treatment population was 86.3% (95% CI: 71.2%–100%) and, in step 2, the percentage in the low-dose group (97.4%, 95% CI: 94.0%–100%) was higher than that in the high-dose group (88.1%, 95% CI: 78.8%–97.5%) ($p=0.04$). The percentage of wound reduction in the total treatment population was 87.3% (95% CI: 76.5%–98.0%) and, in step 2, there was no difference between the low-dose group (77.5%, 95% CI: 60.2%–94.8%) and the high-dose group (88.2%, 95% CI: 69.2%–107.2%) ($p=0.33$). The percentage of granulation area in the total treatment population was 86.3% (95% CI: 73.7%–98.9%) and, in step 2, there was no difference between the low-dose group (96.6%, 95% CI: 92.0%–100%) and the high-dose group (85.4%, 95% CI: 73.6%–97.3%) ($p=0.052$).

Regarding the safety, overall, 13 (76%) of 17 treated patients, comprising 9 (90%) of 10 in the low-dose group and 4 (57%) of 7 in the high-dose group, had at least one adverse reaction. In addition, 6 (60%) in the low-dose group and 2 (29%) in the high-dose group had a reaction that was considered to have a possible, probable, definite, or unknown relationship to the study medication; these reactions are summarized in Table 3. These adverse reactions in the low-dose group were infection (2 reactions), wound complications including wound pain (5 reactions) and wound itching (one reaction), laboratory test abnormality including aspartate aminotransferase increase (one reaction), alanine aminotransferase increase (one reaction), cholinesterase increase (one reaction), and hematoma formation under CGS (one reaction). Adverse reactions in the high-dose group were laboratory test abnormality including hemoglobin decrease (one reaction), C-reactive protein test increase (one reaction), and white blood cell increase (one reaction). One patient in step 1 of the low-dose group discontinued the study because of moderate infection. No serious adverse reactions were reported. Adverse reactions with a clear causal relationship to the study treatment were mild and patients quickly recovered from them.

### *Case presentation*

Case 1: a case of ulcer related to collagen disease of the right leg (step 1, low-dose group). A 35-year-old female had been suffering from rheumatoid arthritis for more than 20 years. She had been treated with oral corticosteroid therapy (7.5 mg/day prednisolone equivalent) and methotrexate. She had a chronic ulcer after trauma on the anterior surface of the leg for 1 month (Fig. 3A). Skin perfusion pressure (SPP) at a site 1 cm proximal to the ulcer was 73 mmHg. After debridement, CGS impregnated with bFGF at 7 $\mu g/cm^2$ was applied and sutured (Fig. 3B, C). On day 14, silicone sheets were removed. The percentage of wound bed improvement was 92.8%. The formed granulation area was evaluated as 93.2% and the ulcer was judged as improved. Three months later, the ulcer was mostly epithelialized (Fig. 3E) and no recurrence was observed 6 months after application (Fig. 3F).

Case 2: a case of venous ulcer of the left leg (step 2, high-dose group). A 77-year-old female had a venous ulcer on her left leg for 1 year after undergoing ligation two times (Fig. 4A). SPP at a site 1 cm proximal to the largest ulcer that was evaluated for safety and efficacy was 65 mmHg. After debridement, CGS impregnated with bFGF at 14 $\mu g/cm^2$ was applied and sutured (Fig. 4B, C). On day 14 after application,

TABLE 3. SUMMARY OF ADVERSE REACTIONS

| | *Low-dose bFGF (n=10)* | *High-dose bFGF (n=7)* | *Total population (n=17)* |
|---|---|---|---|
| Wound infection at studied ulcer | 2 (20%) | 0 | 2 (11.8%) |
| Wound complications | 5 (50%) | 0 | 6 (35.4%) |
| laboratory test abnormality | 3 (30%) | 3 (42.9%) | 6 (35.4%) |
| hematoma formation under CGS | 1 (10%) | 0 | 1 (5.9%) |
| Wound itching | 1 (10%) | 0 | 1 (5.9%) |

Data are *n* (%).
CGS, collagen/gelatin sponge.

the silicone sheets were removed. The wound area and formed granulation area of the largest ulcer were evaluated and the formed granulation area was evaluated as 100%. This ulcer was judged as improved. On day 16, skin grafts were applied on the formed granulation tissues and they took completely (Fig. 4D, E). Two months after application, the ulcers had healed and no recurrence was observed (Fig. 4F).

Case 3: a case of pressure ulcer of the left leg (step 2, high-dose group). A 73-year-old male with type II diabetes had been suffering from left-sided paresthesia after cerebral infarction for 9 years. He had a pressure ulcer on the left lateral malleolus for 6 months. The wound was debrided and treated with negative pressure wound therapy (VAC therapy®, KCI KK, Tokyo, Japan) for 3 weeks, but granulation tissue formation was insufficient and the tendon was still exposed (Fig. 5A). SPP at a site 1 cm proximal to the ulcer was 58.5 mmHg. After debridement, CGS impregnated with bFGF at 14 μg/cm$^2$ was applied (Fig. 5B, C). On day 14, this ulcer was judged as improved (Fig. 5D). On day 23, a skin graft was applied on the formed granulation tissues and it took mostly (Fig. 5D, E). Six months after application, no recurrence was observed (Fig. 5F).

## Discussion

This study is the first clinical trial to evaluate the efficacy and safety of a novel artificial dermis that can perform sustained release of bFGF in the treatment of chronic ulcers.

A bilayered artificial dermis, composed of an upper silicone sheet and a lower collagen sponge, has been used in full-thickness skin defects after injuries or tumor removal.[25]

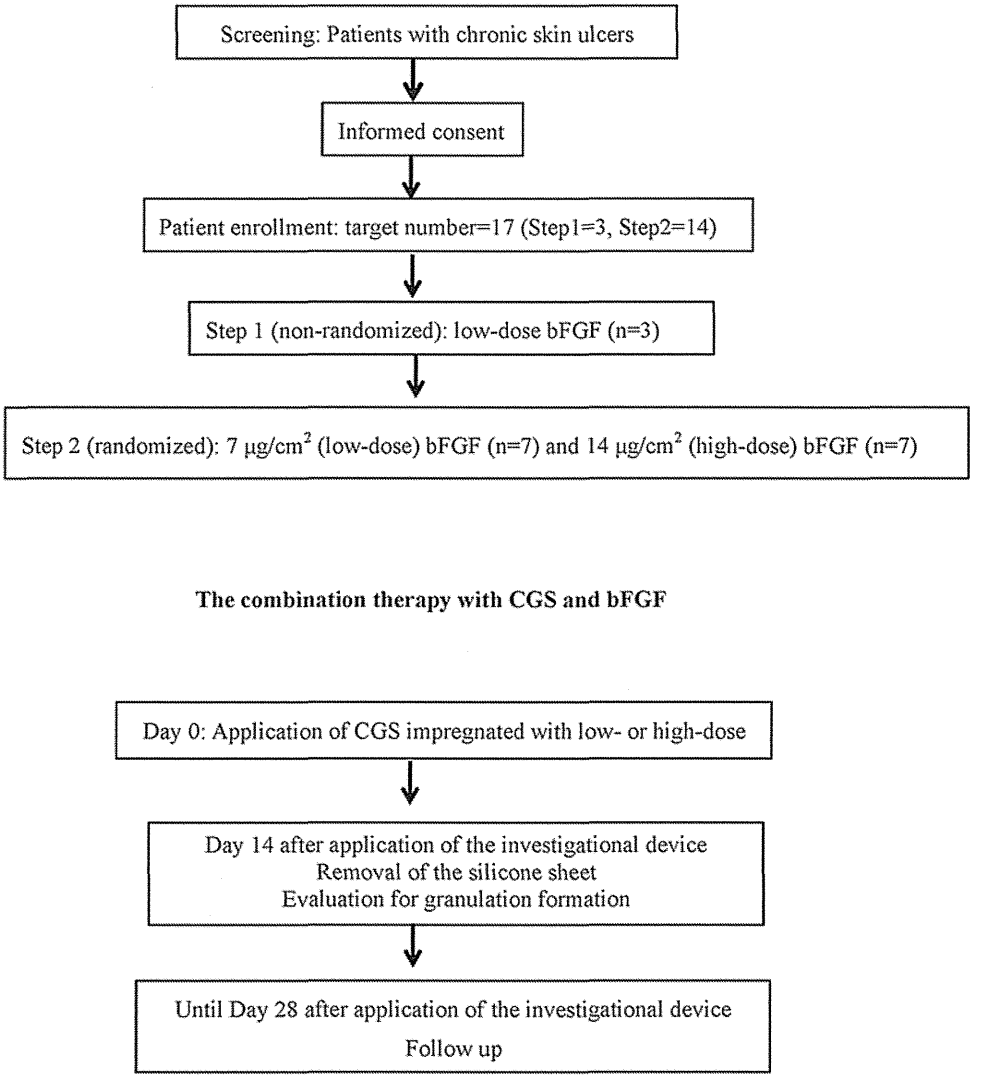


**FIG. 1.** Schema of the study. After enrollment, the initial three patients were enrolled in the low-dose group (step 1) and, after confirming the safety of this step, fourteen patients were randomized to the low-dose or high-dose basic fibroblast growth factor (bFGF) group in step 2. In the combination treatment, collagen/gelatin sponge (CGS) impregnated with bFGF was applied after debridement (day 0). After removing the silicone sheet, the wound area and granulation tissue were evaluated (day 14), and then patients were followed up until day 28.

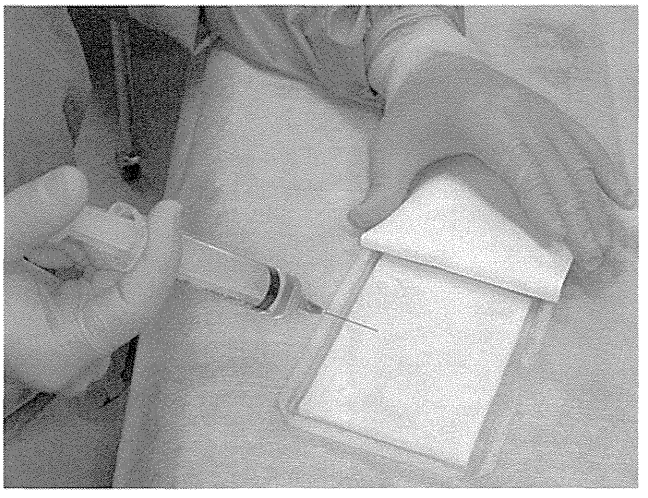

FIG. 2. Impregnation of bFGF solution to CGS. After applying bFGF solution to CGS, CGS was left at room temperature for more than 10 min.

After application, its collagen sponge is biodegraded and replaced by host dermal tissue, so-called dermis-like tissue, in 2 or 3 weeks.[25,26] Secondary split-thickness skin grafting is performed to complete epithelialization on that dermis-like tissue if necessary. When artificial dermis was applied to chronic wounds in this study, dermis-like tissue was not generally expected to be formed because of insufficient blood flow from the wound bed or infection. Therefore, living cell therapy or growth factors will be required for the treatment of chronic wounds. This is the reason why the novel artificial dermis, CGS, which can exhibit sustained release of bFGF, was developed.

Regarding the primary endpoint, wound bed improvement, that of the total treatment population was significantly improved. This primary endpoint was set because it was necessary to evaluate whether or not chronic wounds start to undergo the wound healing process after debridement with our CGS and bFGF treatment. However, in this disease area, a standardized method of evaluating whether a wound begins to heal or not has not been established. The wound area reduction and granulation tissue formation after CGS treatment were focused on because wound area reduction within

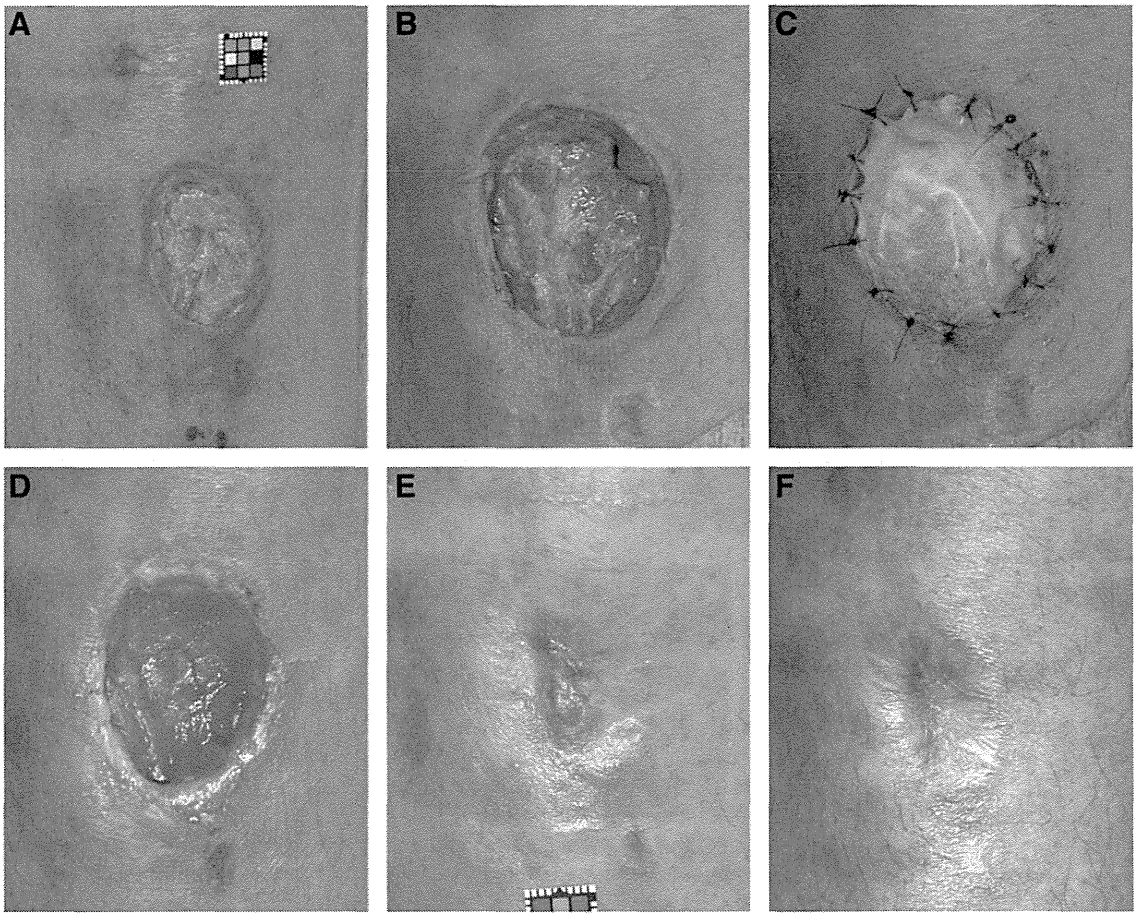


FIG. 3. A case of ulcer related to collagen disease of the right leg (step 1, low-dose group). **(A)** This patient had a traumatic ulcer and granulation tissue was not formed with conventional treatments. **(B)** Necrotic tissue and marginal skin of ulcer were debrided (day 0). The baseline wound area was 532.7 $mm^2$. **(C)** CGS impregnated with bFGF was applied and sutured to wounds (day 0). **(D)** On day 14, the ulcer area was 562.0 $mm^2$ and the granulated area was evaluated as 523.7 $mm^2$. A small area colored yellow was not granulated. The percentage of wound bed improvement was 92.8%. **(E)** Three months later, the ulcer was mostly epithelialized. **(F)** Six months after application, no recurrence was observed. Color images available online at www.liebertpub.com/tea