fore, TT expression did not appear to be associated with the IFN response in patients with *ss469415590* TT/TT. Prokunina-Olsson *et al.* suggest that the intracellular function of IFNL4 may be important for its antiviral activity [19]. Association between antiviral *IFNL4* ΔG expression and SVR rate in recipients with the *ss469415590* risk ΔG allele (Fig. 2c) suggests that recipient IFNL4 also functions intramacrophage or other immune cell. We also measured *IFNL4* expression in liver grafts. However, the expression levels in the majority of grafts were under the detection limit at our institute (data not shown).

ISG15 and *USP18* have been reported to inhibit IFN activity [16–18], and the expression of ISGs has been reported as a predictor of the possible response to IFN therapy [23,24]. Therefore, we measured *ISG15* and *USP18* expression in the recipient liver resected at LDLT and analysed the correlation between these ISGs and IFNL4 expression. *ISG15* and *USP18* expression levels were also correlated with *IFNL4* ΔG expression (Fig. 3c). These ISGs' expression levels would be induced by *IFNL4*. The results indicate that the *rs8099917* polymorphism risk allele reflects the presence of the antiviral *IFNL4* gene and that ISGs' expression levels are affected by *IFNL4* expression levels. The IFN therapeutic response after LT could be predicted by measuring *IFNL4* ΔG expression. *ISG15* and *USP18* expression levels were significantly lower in the recipients who achieved SVR to IFN therapy after LDLT than the recipients who did not achieved SVR (Fig. 3b). However, in this study, majority of the recipients with SVR had possessed *IFNL4 ss469415590* TT/TT genotype. Therefore, this population bias may affect the difference of ISGs expression levels. Alternatively, correlations between ISG expression levels in the liver and peripheral blood mononuclear cells [25] suggest that ISG and IFNL4 in macrophages or other immune cells also inhibit the IFN response. We could were not able to analyse *IFNL4* expression at the time of pretreatment of IFN. The expression levels in peripheral blood mononuclear cells would be beneficial in helping to predict the response to IFN therapy more accurately.

Recently, a combination of telaprevir, HCV protease inhibitor and IFN therapy has been shown to improve anti-HCV treatment in Japan [26,27]. However, telaprevir treatment after LT requires considerable attention because of trough concentrations of telaprevir and calcineurin inhibitor or the presentation of adverse events [28,29]. Several other anti-HCV direct-acting antiviral (DAA) treatments are currently being developed in clinical trials [30,31]. A combination of IFN/DAA or DAA/DAA therapy can achieve SVR in chronic hepatitis C patients, even in those patients displaying the *IL28B* unfavourable genotype [32–35]. Regimes involving DAA should also improve the outcome of treatment for recipients with the *IL28B* and *IFNL4* unfavourable genotype after LT. Therefore, cases that possess these risk alleles in either transplant or liver graft recipients may wish to choose to await approval of new drugs.

IL28B and *IFNL4* genotyping will be important in the future to develop and progress individualized therapy to HCV infections, rather than just predicting the current therapeutic response.

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STATEMENT OF INTERESTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1: Association of *IFNL4* and *IL28B* genetic polymorphisms with sustained virological response (SVR) rate to interferon and ribavirin combination therapy after liver transplantation. Sustained virological response rates in recipients and donors with indicated *IFNL4 ss469415590* (left graph) or *IL28B rs8099917* (right graph) polymorphisms.

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Decreased immunoglobulin G levels after living-donor liver transplantation is a risk factor for bacterial infection and sepsis

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Abstract: *Background.* Several studies have suggested an association between post-transplant immunoglobulin (Ig) levels and the development of infection in solid organ transplantation. We therefore conducted exploratory analyses of potential factors associated with bacterial infection/sepsis after living-donor liver transplantation (LDLT).
Methods. Blood samples from 177 recipients who received primary LDLT between September 1999 and November 2011 were available for study. Hypogammaglobulinemia was defined as having at least 1 IgG level <650 mg/dL within 7 days after LDLT. Risk factors for developing post-transplant bacterial infection and sepsis within 3 months after LDLT were analyzed.
Results. Fifty (28.2%) recipients experienced bacterial infection within 3 months of LDLT. Eighty-four (47.5%) recipients had hypogammaglobulinemia, although no recipients had hypogammaglobulinemia before LDLT. Hypogammaglobulinemia, undergoing hepaticojejunostomy, and portal pressure at closure >15 mmHg were independent risk factors for developing bacterial infection within 3 months of LDLT ($P < 0.0001$ $P = 0.0008$, and $P = 0.011$, respectively). The odds ratio (OR) and confidence interval (CI) for hypogammaglobulinemia were 4.79 and 2.27–10.7, respectively. Twenty-four (13.6%) recipients developed bacterial sepsis within 3 months. Hypogammaglobulinemia, operative time >14 h, model for end-stage liver disease score >15, and no mycophenolate mofetil use were independent risk factors for developing bacterial sepsis ($P = 0.009$, $P = 0.001$, $P = 0.003$, and $P = 0.005$, respectively). The OR and CI for hypogammaglobulinemia were 3.83 and 1.38–12.0, respectively.
Conclusions. Hypogammaglobulinemia within 7 days of LDLT was a significant risk factor for post-transplant bacterial infection and sepsis.

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Key words: living-donor liver transplantation; hypogammaglobulinemia; sepsis

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Immunoglobulin G (IgG) is synthesized by B cells, and IgG levels are elevated in patients with liver cirrhosis as a non-specific response to bacteremia, increased Ig production, or portal systemic shunting. Increased numbers of plasma cells in the bone marrow and liver may be the source of increased IgG (1). Previous studies suggested an association between post-transplant Ig levels and the development of infection in solid organ transplantation (2, 3). Most studies focused on cytomegalovirus or opportunistic infection at a relatively late term after organ transplantation (4, 5). However, very few studies have focused on bacterial

Abbreviations: CI, confidence interval; CT, computed tomography; CyA, cyclosporine; FHF, fulminant hepatic failure; GW, graft weight; IgG, immunoglobulin G; IVIG, intravenous immunoglobulin; LDLT, living-donor liver transplantation; LL+C, left lobe with caudate lobe; LT, liver transplantation; MELD, model for end-stage liver disease; MMF, mycophenolate mofetil; OR, odds ratio; POD, postoperative day; SFS, small-for-size; SLW, standard liver weight.

sepsis and Ig levels in the early phase after liver transplantation (LT). Following the release of a report in 1994 that demonstrated successful living-donor liver transplantation (LDLT) between adults, living donors have been increasingly used because of the disparity between demand and supply of deceased donors (6). However, to our knowledge, no published reports have described the incidence and impact of IgG levels before and after LDLT. The small graft size is the main disadvantage of adult-to-adult LDLT, because it results in increased portal venous pressure, impaired bowel motility, bacterial translocation, ascites production, and hyperbilirubinemia, although it does not necessarily lead to graft loss (7). Recently, we reported the impact of bacterial sepsis on the survival of patients who received LDLT (8). Intraoperative blood loss >10 L and no enteral feeding started within 48 h after LDLT were independent risk factors for bacterial sepsis. No data for IgG levels have been reported. Therefore, we retrospectively analyzed IgG levels from stored blood specimens in an effort to assess whether a decreased IgG level (hypogammaglobulinemia) was an independent risk factor for developing bacterial infection and sepsis after LDLT. The aim of this study was to explore the impact of decreased IgG levels on developing bacterial infection and sepsis after LDLT.

Study design

We conducted exploratory analyses of potential factors associated with bacterial infection and sepsis after LDLT. Stored samples from 177 recipients who received primary LDLT between September 1999 and November 2011 were available and were used in the study. IgG levels and potential factors associated with infection and sepsis, such as operative time, blood loss, model for end-stage liver disease (MELD) score, and graft weight (GW), were retrospectively collected from the database and analyzed.

Patients and methods

Patients

Graft types included left lobe with caudate lobe (LL+C) graft (n = 100), right lobe graft without the middle hepatic vein (n = 73), and posterior segment graft (n = 4). The etiology of liver cirrhosis was hepatitis C (n = 88), primary biliary cirrhosis (n = 22), fulminant hepatic failure (FHF, n = 16), hepatitis B (n = 17), alcohol abuse (n = 9), cryptogenic (n = 8), primary sclerosing cholangitis (n = 6), autoimmune hepatitis (n = 5), Wilson's disease (n = 2), biliary atresia (n = 1), Alagille syndrome (n = 1), hemangioma (n = 1), and epithelioid hemangio endothelioma (n = 1) (Table 1). ABO incompatible cases who received exogenous intravenous Ig (IVIG) to prevent humoral rejection (9) were excluded. Serum samples were collected before LDLT, on postoperative day (POD) 1, POD 3, and POD 7, and were used for IgG measurement.

Donor and graft selection

Donors were selected from candidates who volunteered to be living donors (6, 10). Donors were required to be within the third degree of consanguinity with recipients or spouses, and to be between 20 and 65 years of age. For a donor who was not within the third degree of consanguinity, individual approval was obtained from the Ethics Committee of Kyushu University Hospital. Altruistic donations were not used.

Eligible donors underwent imaging studies, including chest and abdominal x-rays and 3-mm-slice computed tomography (CT) scans for graft volumetric analysis. Three-dimensional CT was introduced for volumetric analysis and delineation of vascular anatomy. The standard liver weight (SLW) of recipients was calculated according to the formula of Urata et al. (11). GW was predicted by CT volumetric analysis. Decisions regarding graft type for recipients were based on the preoperatively predicted GW-to-SLW ratio. LL+C graft was used when the preoperatively predicted GW-to-SLW ratio was >35%. When GW-to-SLW ratio with LL+C graft was <35% and remnant donor liver volume after right lobectomy was >35%, a right lobe graft was used. Posterior segment graft was considered when the donor's vascular anatomy was suitable to accept a posterior segment. We previously reported a formula (7), which consisted of GW-to-SLW ratio, MELD score, donor age, and the presence of huge portcaval shunt, to predict early graft function. This formula was not fully used to select the graft type during this study.

Surgery and postoperative management

The graft retrieval technique, recipient surgery, and perioperative management of the recipients, including immunosuppression regimens were described previously (12). Simultaneous splenectomy was performed in 95 recipients for decreasing portal vein pressure or for improving pancytopenia (13). Five recipients

Risk factors for developing infection within 3 months after living-donor liver transplantation (LDLT): univariate analysis

Variables	Total (n = 177)	Infection within 3 months		P-value
		Yes (n = 50)	No (n = 127)	
Recipient				
Gender (Male/Female, %)	49.7/50.3	48.0/52.0	50.4/49.6	0.77
Age (years)	53.7 ± 10.8	52.9 ± 11.4	54.0 ± 10.5	0.57
Etiology				
Postnecrotic cirrhosis (%)	127 (71.8)	35 (70.0)	92 (72.5)	0.56
Cholestatic cirrhosis (%)	30 (16.9)	10 (20.0)	20 (15.7)	
Fulminant hepatic failure (%)	16 (9.0)	3 (6.0)	13 (10.2)	
Others (%)	4 (2.3)	2 (4.0)	2 (1.6)	
IgG pre-LDLT	2316 ± 823	2356 ± 840	2230 ± 820	0.68
IgG on POD 1	820 ± 331	731 ± 265	855 ± 348	0.023
IgG on POD 3	856 ± 350	784 ± 333	884 ± 354	0.087
IgG on POD 7	781 ± 327	687 ± 251	818 ± 346	0.020
Hypogammaglobulinemia (Yes/No, %)	47.5/52.5	72.0/28.0	37.8/62.2	<0.0001
MELD score	14.4 ± 7.1	15.4 ± 7.7	14.0 ± 6.9	0.26
Pre-LDLT ICU bound (Yes/No, %)	10.2/89.8	12.0/88.0	9.4/90.6	0.61
Diabetes mellitus (Yes/No, %)	16.9/83.1	18.0/82.0	16.5/83.5	0.82
Operative time >14 h (Yes/No, %)	31.6/68.4	46.0/54.0	26.0/74.0	0.009
Operative blood loss >10 L (Yes/No, %)	8.5/91.5	18.0/82.0	4.7/95.3	0.005
Biliary reconstruction (D-D/H-J, %)	88.1/11.9	74.0/26.0	93.7/6.3	0.0003
Splenectomy (Yes/No, %)	58.8/41.2	54.0/46.0	60.6/39.4	0.42
Portal pressure at closure (mmHg) >15 (Yes/No, %)	56.5/43.5	72.0/28.0	50.4/49.6	0.009
CNI (TAC/CyA/None, %)	55.9/42.9/1.2	56.0/40.0/4.0	55.9/44.1/0	0.77
MMF use (Yes/No, %)	81.9/10.1	78.0/22.0	83.5/16.5	0.40
Enteral nutrition started within 48 h (Yes/No, %)	51.4/48.6	44.0/56.0	54.3/45.7	0.22
Donor				
Gender (Male/Female, %)	66.1/33.9	74.0/26.0	63.0/37.0	0.16
Age (years)	35.2 ± 10.9	36.1 ± 11.8	34.8 ± 10.5	0.49
Graft (Left/Right/Posterior, %)	56.5/41.2/2.3	62.0/34.0/4.0	54.3/44.1/1.6	0.33
GW-to-SLW ratio (%)	41.5 ± 8.2	41.1 ± 7.8	41.6 ± 8.3	0.74
ABO (identical/compatible/incompatible)	71.8/22.6/5.6	78.0/20.0/2.0	69.3/23.6/7.1	0.33

IgG, immunoglobulin G; POD, postoperative day; MELD, model for end-stage liver disease; ICU, intensive care unit; D-D/H-J, duct-to-duct/hepaticojejunostomy; CNI, calcineurin inhibitor; TAC, tacrolimus; CyA, cyclosporine; MMF, mycophenolate mofetil; GW, graft weight; SLW, standard liver weight.

Table 1

underwent splenectomy before LDLT. Since 2001, duct-to-duct anastomosis has been preferred to Roux-en-Y hepaticojejunostomy for bile duct reconstruction (14). Hepaticojejunostomy was performed when duct-to-duct anastomosis could not be applied, such as in those with biliary atresia or primary sclerosing cholangitis. Duct-to-duct or hepaticojejunostomy was performed over a 2.0-mm C-tube with intermittent 6-0 PDS-II sutures (8, 14). Perioperative anti-microbial prophylaxis consisted of IV cefotaxime (4 g/day) and ampicillin sulbactam (6 g/day) 4 times/day for 3 days after LDLT, and was started 30 min before surgery. Once bacterial sepsis was clinically suspected, broad-spectrum antibiotics were administered empirically (8). IVIG was not

exogenously infused until the development of sepsis. Immunosuppression was initiated using a protocol based on either tacrolimus (Prograf; Astellas Pharma Inc., Tokyo, Japan) or cyclosporine (CyA) (Neoral; Novartis Pharma K.K., Tokyo, Japan) with steroids. Tacrolimus was used in 108 recipients, and CyA in 90 recipients. Two recipients did not receive calcineurin inhibitors owing to poor postoperative course. A target trough level of tacrolimus was set at 10 ng/mL for 3 months after LDLT, followed by 5–10 ng/mL thereafter. A target trough level of CyA was set at 250 ng/mL for 3 months after LDLT, followed by 150–200 ng/mL thereafter. Methylprednisolone was initiated on the day of LDLT, tapered, and converted to prednisolone 7 days after LDLT. Prednisolone treatment was tapered and discontinued 6 months after LDLT. Mycophenolate mofetil (MMF) was used in 145 recipients and was started at 1 g/day on the day after LDLT, tapered, and discontinued until 6 months after LDLT. A trough level was not measured for MMF. All patients had monthly follow-ups, and the median follow-up period was 1491 days, with 700 days and 2345 days as the 25th and 75th percentiles, respectively.

Post-LDLT infection and bacterial sepsis

Incidence of bacterial sepsis was set as the primary end-point. Bacterial sepsis was defined as the isolation of bacteria other than common skin contaminants from a single blood culture within 3 months after LDLT, along with clinical symptoms, including high fever, shivering, dyspnea, altered mental status, tachycardia, or hypotension (8). Infections included pneumonia, cholangitis, peritonitis, urinary tract infection, and wound infection. Pneumonia was defined as the isolation of bacteria from cultured sputum accompanied by radiological infiltration. Cholangitis was defined when patients had clinical symptoms including high fever, right upper quadrant pain, and elevated serum biliary enzymes. Peritonitis was defined as the isolation of bacteria from ascites culture and clinical symptoms including abdominal pain and fever. The definition of urinary tract infection was the isolation of bacteria from urine culture, along with urodynia or pollakiuria. Wound infection was defined as the isolation of bacteria from culture of effusion from skin redness.

Statistical analysis

Hypogammaglobulinemia was defined as having at least 1 IgG level <650 mg/dL (15) within 7 days after LDLT. Data were expressed as means ± standard deviation. Logistic regression analysis was applied to the multivariate analyses. Variables that were used for the univariate analysis included recipient age, donor age, GW-to-SLW ratio, MELD score, the presence of diabetes mellitus, recipient gender, donor gender, Intensive Care Unit stay before LDLT, serum IgG level before and after LDLT, blood loss during LDLT, bile duct reconstruction method, graft type, tacrolimus or CyA use, and MMF use. Multiple logistic regression analysis was performed with selected predictors by a stepwise procedure from variables with a *P*-value <0.10 by univariate analysis. The significance levels for the procedure were 0.05, to add variables into the method, and 0.08, to keep variables in the model. All statistical analyses were performed using JMP 9.0 software (SAS, Inc., Cary, North Carolina, USA).

Results

In this study, 50 (28.2%) recipients experienced bacterial infection after LDLT. Table 1 shows the characteristics of the recipients and donors. Mean serum IgG levels of pre-LDLT, POD 1, POD 3, and POD 7 were 2316 ± 823, 820 ± 331, 856 ± 350, and 781 ± 327, respectively. Serum IgG levels at POD 1, POD 3, or POD 7 were significantly lower compared with that of pre-LDLT ($P < 0.0001$). Serum IgG levels of pre-LDLT in patients with cirrhosis (2393 ± 727) were significantly higher than in patients with FHF (1469 ± 327, $P < 0.0001$). Serum levels of IgG at POD 1, POD 3, or POD 7 were not different between recipients with cirrhosis and recipients with FHF. Finally, 84 (47.5%) recipients developed hypogammaglobulinemia within 7 days after LDLT, although no recipients had hypogammaglobulinemia before transplantation. Risk factors for developing hypogammaglobulinemia were a preoperative value of IgG ($P = 0.002$), operative bleeding ($P = 0.003$), and operative time ($P = 0.002$). Mean operative bleeding in recipients with hypogammaglobulinemia was significantly greater compared with recipients without hypogammaglobulinemia (5525 mL vs. 3618 mL). Recipients with hypogammaglobulinemia had a longer operative time compared with recipients without hypogammaglobulinemia (832 min vs. 755 min). Univariate analysis revealed that recipients who experienced bacterial infection within 3 months after LDLT developed hypogammaglobulinemia, had an operative time >14 h, had more operative blood loss, were more likely to have received hepaticojejunostomy for biliary reconstruction, and had high portal venous pressure at the end of LDLT (Table 1).

Risk factors for developing infection within 3 months after living-donor liver transplantation: multivariate analysis

Variables	Odds ratio	95% CI	*P*-value
Hypogammaglobulinemia	4.79	2.27–10.7	<0.0001
Hepaticojejunostomy	5.81	2.06–17.7	0.0008
Portal vein pressure at closure >15 mmHg	2.63	1.24–5.85	0.011

CI, confidence interval.

Table 2

The selected variables as predictors in the model by a stepwise procedure were hypogammaglobulinemia, receiving hepaticojejunostomy, and portal pressure at closure >15 mmHg. Multivariate logistic regression analysis revealed that hypogammaglobulinemia (odds ratio [OR]; 4.79, $P < 0.0001$), receiving hepaticojejunostomy (OR; 5.81, $P = 0.0008$), and portal pressure at closure >15 mmHg (OR; 2.63, $P = 0.011$) were independent risk factors for developing bacterial infection within 3 months after LDLT in this study (Table 2).

Twenty-four (13.6%) recipients developed bacterial sepsis within 3 months after LDLT. The mean onset day was POD 16 (range 4–84 days).

Univariate analysis revealed that hypogammaglobulinemia, receiving hepaticojejunostomy, portal pressure at closure >15 mmHg, MELD score >15, operative time >14 h, operative blood loss >10 L, and no MMF use were risk factors for developing bacterial sepsis within 3 months after LDLT ($P = 0.004$, $P = 0.005$, $P = 0.049$, $P = 0.025$, $P = 0.0005$, $P = 0.002$, and $P = 0.037$, respectively) (Table 3). The selected variables as predictors in the model by a stepwise procedure were hypogammaglobulinemia, operative time >14 h, MELD score >15, and no MMF use. Multivariate analysis revealed that hypogammaglobulinemia (OR; 3.83, $P = 0.009$), an operative time >14 h (OR; 5.17, $P = 0.001$), MELD score >15 (OR; 5.67, $P = 0.003$), and no MMF use (OR; 5.49, $P = 0.005$) were independent risk factors for developing bacterial sepsis within 3 months after LDLT (Table 4).

Discussion

This is the first report to our knowledge to identify a correlation between serum IgG levels and bacterial sepsis development after LDLT. Multivariate analysis revealed that hypogammaglobulinemia within 7 days after LDLT was an independent risk factor for developing bacterial sepsis, which caused higher mortality rates. Data from this study demonstrated that 6-month survival in patients who developed bacterial sepsis within 3 months ($n = 24$) was significantly worse than in patients who did not develop sepsis ($n = 153$, $P < 0.0001$, data not shown). Monitoring IgG levels may aid in clinical management of LDLT recipients (3). The present study suggested that IgG levels dramatically decreased during surgery. Patients with liver failure can develop major coagulation abnormalities, splenomegaly, portal hypertension, and nutritional deficiencies can result in associated thrombocytopenia (16). Such coagulopathy sometimes causes massive bleeding during LT and can cause longer surgery time. Thus, recipients with hypogammaglobulinemia had more operative bleeding and longer operative times compared with recipients without hypogammaglobulinemia. This suggested that IgG levels were reduced during surgery, as well as after surgery owing to increased capillary permeability and increased catabolism.

This study had some limitations including the use of stored samples. In addition, IgG concentrations were not measured at the onset of sepsis. Furthermore, we divided septic recipients according to the presence of hypogammaglobulinemia within 7 days after LDLT, but no significant difference was seen in any variables between patients with hypogammaglobulinemia and patients without hypogammaglobulinemia. This result may have been because of the small number of patients in both groups (data not shown). In addition, samples were only obtained from half of the patients in the study. These concerns might lead to a potential bias. Further additional studies are required to explain these concerns fully.

Hypogammaglobulinemia had a negative impact on the development of infections in patients undergoing heart (5) or lung (17) transplantation. Size mismatch is a major obstacle in LDLT between adults, and small-for-size (SFS) graft syndrome after LDLT remains a major complication of the procedure. Most surgeons believe that SFS graft syndrome can induce postoperative hyperbilirubinemia, intractable ascites, and prolonged coagulopathy, which ultimately lead to septic complication and liver failure (7, 13). Polyclonal IVIG can modulate the host immune response and may improve outcomes in patients who develop septic shock (15). IVIG can neutralize endotoxins, limit the production of cytokines, increase serum bactericidal activity, and block the complement cascade (18, 19). IVIG has been administered to various categories of patients regardless of their baseline IgG levels. This approach carries the possibility of using IVIG in patients with normal

Risk factors for developing sepsis within 3 months after living-donor liver transplantation (LDLT): univariate analysis

Variables	Sepsis within 3 months		
	Yes (n = 24)	No (n = 153)	P-value
Recipient			
Gender (Male/Female, %)	41.7/58.3	51.0/49.0	0.40
Age >55 years (Yes/No, %)	66.7/33.3	77.1/22.9	0.27
Etiology			
Postnecrotic cirrhosis (%)	16 (66.7)	111 (72.5)	0.20
Cholestatic cirrhosis (%)	4 (16.7)	26 (17.0)	
Fulminant hepatic failure (%)	2 (8.3)	14 (9.2)	
Others (%)	2 (8.3)	2 (8.3)	
Hypogammaglobulinemia (Yes/No, %)	75.0/25.0	43.1/56.7	0.004
MELD score >15 (Yes/No, %)	37.5/62.5	17.6/83.3	0.025
Pre-LDLT ICU bound (Yes/No, %)	20.8/79.2	8.5/91.5	0.063
Diabetes mellitus (Yes/No, %)	16.7/83.3	17.0/83.0	0.97
Operative time >14 h (Yes/No, %)	62.5/37.5	26.8/73.2	0.0005
Operative blood loss >10 L (Yes/No, %)	25.0/75.0	5.9/94.1	0.002
Biliary reconstruction (D-D/H-J, %)	70.8/29.2	90.8/9.2	0.005
Splenectomy (Yes/No, %)	54.2/45.8	59.5/40.5	0.62
Portal pressure at closure >15 mmHg (Yes/No, %)	75.0/25.0	53.6/46.4	0.049
CNI (TAC/CyA/None, %)	66.7/25.0/8.3	54.2/45.8/0	0.102
MMF use (Yes/No, %)	66.7/33.3	84.3/15.7	0.037
Enteral nutrition started within 48 h (Yes/No, %)	37.5/62.5	53.6/46.4	0.14
Donor			
Gender (Male/Female, %)	75.0/25.0	64.7/35.3	0.32
Age >45 years (Yes/No, %)	37.5/62.5	21.6/78.4	0.088
Graft (Left/Right/Posterior, %)	54.2/37.5/8.3	56.9/41.8/1.3	0.098
GW-to-SLW ratio >40% (Yes/No, %)	66.7/33.3	49.0/51.0	0.11
ABO (identical/compatible/incompatible, %)	79.2/20.8/0	70.6/22.9/6.5	0.40

MELD, model for end-stage liver disease; ICU, intensive care unit; D-D/H-J, duct-to-duct/hepaticojejunostomy; CNI, calcineurin inhibitor; TAC, tacrolimus; CyA, cyclosporine; MMF, mycophenolate mofetil; GW, graft weight; SLW, standard liver weight.

Table 3

IgG levels who would not benefit from a further increase in plasma IgG concentrations. Monitoring IgG level could help to select recipients that have developed sepsis who might benefit from IVIG administration. A preemptive use of IVIG replacement may serve as a new strategy for managing LDLT recipients with hypogammaglobulinemia. Thus, a prospective study is necessary to evaluate the impact of IVIG on recipients with hypogammaglobulinemia after LDLT.

Splenectomy did not cause hypogammaglobulinemia, infection, or sepsis in this study. Previous studies suggested that splenectomy in liver transplantation is closely associated with septic complications and a poorer prognosis, because the spleen is a huge source of B cells (20, 21). Splenectomy is commonly performed at our medical center, as we reported favorable outcomes of splenectomy for overcoming SFS graft syndrome in LDLT recipients (13, 22). IgG levels in recipients a long time after LDLT with splenectomy may decrease compared with recipients who did not undergo the procedure. Further immunological studies are required to determine how splenectomy affects the incidence of hypogammaglobulinemia in liver transplantation.

Risk factors for developing sepsis within 3 months after living-donor liver transplantation: multivariate analysis

Variables	Odds ratio	95% CI	*P*-value
Hypogammaglobulinemia	3.83	1.38–12.0	0.009
Operative time >14 h	5.17	1.94–14.9	0.001
MELD score >15	5.67	1.86–18.2	0.003
No MMF use	5.49	1.71–18.3	0.005

CI, confidence interval; MELD, model for end-stage liver disease; MMF, mycophenolate mofetil.

Table 4

In conclusion, nearly half of the recipients in this study developed hypogammaglobulinemia within 7 days after LDLT. Hypogammaglobulinemia was a risk factor for post-transplant infection and sepsis. A prospective study in LDLT is necessary to evaluate the impact of IVIG for recipients with hypogammaglobulinemia after LDLT.

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Regular Article

Efficacy of postoperative anticoagulation therapy with enoxaparin for portal vein thrombosis after hepatic resection in patients with liver cancer

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ABSTRACT

Backgrounds: Enoxaparin, low-molecular-weight heparin, has become a routine thromboprophylaxis in general surgery.
Study design: A retrospective cohort study was performed in 281 patients who underwent hepatic resections for liver cancers from 2011 to 2013. These patients were divided into two groups; an enoxaparin (-) group (n = 228) and an enoxaparin (+) group (n = 53). Short-term surgical results including venous thromboembolism (VTE) and portal vein thrombosis (PVT) were compared.
Results: In the enoxaparin (+) group, the patients' age (65 *vs.* 69 years; p = 0.01) and BMI (22.9 *vs.* 24.4; p < 0.01) were significantly higher. According to the symptomatic VTE, symptomatic pulmonary embolism occurred in one patient (0.4%) in the enoxaparin (-) group, but the complication rate was not significantly different (p = 0.63). The complication rate of PVT was significantly lower in the enoxaparin (+) group (10 *vs.* 2%; p = 0.04). The independent risk factors for PVT were an operation time ≥ 300 minutes (Odds ratio 6.66) and non-treatment with enoxaparin (Odds ratio 2.49).
Conclusions: Postoperative anticoagulant therapy with enoxaparin could prevent PVT in patients who underwent hepatic resection for liver cancers.

Introduction

Venous thromboembolism (VTE) represented by pulmonary embolism (PE) or deep venous thrombosis (DVT) is a significant cause of morbidity and mortality in patients undergoing gastrointestinal surgery for malignancy, and pharmacologic prophylaxis is important [1,2]. One of the major cautions regarding pharmacologic prophylaxis is the risk of major bleeding complication, but a recent systemic review reported that bleeding requiring a change of care occurs in less than 3% of cases [3]. It is well known that several hemostatic alternations are present in patients with liver disease; primary hemostasis is often impaired due to thrombocytopenia and secondary hemostasis can be hampered by the reduced synthesis of coagulation factors [4].

Meta-analysis of the use of low-molecular-weight heparin (LMWH) such as enoxaparin in the prevention of venous thromboembolism in general surgery clearly demonstrates that LMWH is associated with lower rates of VTE than elastic compression without compromising patient safety, and similar safety and efficacy in preventing VTE to unfractionated heparin (UFH) [5]. In Japan, two randomized studies demonstrated that 20 mg enoxaparin taken twice daily has a good safety profile and is effective for the prevention of VTE in patients undergoing total hip and knee replacement [6] and abdominal or pelvic cancer surgery [7].

LMWH has potential advantageous properties such as two-fold or three-fold longer plasma half-life when compared with commercially available UFH at therapeutic doses, and a 90-95% bioavailability following subcutaneous administration [8]. These advantageous properties of LMWH obviate the need for serum concentration monitoring and enable single or double daily dosing [8]. LMWH also showed decreased interaction with platelets, and a significantly lower complication rate (0/333 patients) of heparin-induced thrombocytopenia (HIT) than UFH (9/332 patients) (0 vs. 2.7%; p = 0.0018) [9].

Another possible agents for postoperative anticoagulation therapy against VTE is the synthetic factor Xa inhibitor fondaparinux: a randomized clinical trial reported that postoperative fondaparinux (4.6% for VTE) was at least as effective as LMWH (6.1% for VTE) in patients undergoing high-risk abdominal surgery [10]. For prevention against hemorrhagic complications after liver surgery under anticoagulant therapy, we prefer enoxaparin because it has a neutralizer such as protamine.

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Portal vein thrombosis (PVT) is a potentially life-threatening complication that occurs after hepatobiliary pancreatic surgery [11,12]. Theoretically, splanchnic vein thrombosis such as PVT cannot be prevented by mechanical prophylaxis by elastic compression leg stockings and/or intermittent pneumatic compression (IPC). PVT was reported to occur in 12 of 22 (55%) patients who underwent laparoscopic splenectomy [13]. Recently, we reported postoperative PVT after hepatic resection occurred in 19 of 208 patients (9.1%), and closely related to delayed recovery of liver function and delayed liver regeneration [14]. Therefore, making an accurate diagnosis and rapidly initiating treatment for PVT are indispensable. However, there are no detailed reports about prophylaxis against PVT after hepatic resection. Accurate anticoagulation drug therapy could prevent PVT after hepatic resection.

We herein report a series of consecutive patients who underwent hepatic resection for liver cancers with or without postoperative enoxaparin administration. We examined the clinical efficacy of enoxaparin for prevention of VTE and PVT.

Methods

Patients

During the 3 years from 2011 through 2013, 287 hepatic resections for liver cancers were performed at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Six patients were excluded from this study, 3 because they had low platelet counts $\leq 10 \times 10^4/\mu l$, 2 because they had low preoperative % prothrombin time (PT) $\leq$ 70%, and one who received perioperative UFH for strict anticoagulant therapy because of a mechanical cardiac valve. Therefore, 281 patients were included in this study of the clinical efficacy of anticoagulant therapy with enoxaparin. The pathological diagnoses for liver tumors of patients in this series were as follows: 181 hepatocellular carcinoma (HCC), 25 intrahepatic cholangiocarcinomas (ICC), 2 cystadenocarcinoma, 1 sarcoma, and 72 metastatic liver cancers (59 colorectal liver metastasis). All patients undergoing hepatic resection had an Eastern Cooperative Oncology Group Performance status of 0-2.

Perioperative mechanical thromboprophylaxis by elastic compression legs stockings and IPC were applied to all patients. From 2011 to 2012, an anticoagulant drug was administered according to the judgment of each patient's physician in charge. From April 2013 on, patients were routinely administered enoxaparin. All 281 patients were divided into 2 groups the enoxaparin (-) group (n = 228), which also had no anticoagulant drug such as UFH or fondaparinux, and the enoxaparin (+) group (n = 53).

Surgical Techniques and Peri-operative Management

Details of our surgical techniques and patient selection criteria for hepatic resection against HCC, ICC, and CRM have been reported previously [15–17]. The key factor concerning the indication for hepatic resection is "remnant liver function" to avoid the fatal postoperative liver failure, and patients with an indocyanine green dye retention rate at 15 minutes (ICGR-15) $\leq$ 40% were selected for hepatic resection [15]. To stabilize the coagulation and fibrinolysis in hepatic resection, 200 mg nafamostat mesilate was given daily, both during and up to 2 days after operation [18], and preoperative steroid (500 mg methylpredonisolone) administration was routinely performed [19]. Intravenous antibiotics for surgical prophylaxis were also given for 2 days after operation.

In almost all hepatic resections, intermittent Pringle's maneuvers, consisting of clamping the portal triad for 15 minutes and then releasing the clamp for 5-minute intervals, or hemivascular occlusions [20] were applied intraoperatively. The CUSA system (Valley Lab, Boulder, CO, USA) has been used with addition of a VIO soft-coagulation system (ERBE Elektromedizin, Tübingen, Germany) [21]. Hepatic venous backflow control [22], which was typically achieved extrahepatically before dividing the liver, and Belghiti's hanging maneuver [23], where a tape was introduced behind the caudate lobe through the groove between the right and middle hepatic vein, were performed as necessary, especially in major hepatic resection. An intraoperative bile leakage test was routinely performed to prevent the postoperative bile leakage [24]. Laparoscopic hepatic resections in the semiprone position were applied to 37 patients in this series [25]. In patients with open hepatic resections (n = 219), an epidural catheter was inserted until the 2nd postoperative day; those with laparoscopic hepatic resection (n = 62) did not receive an epidural catheter and were not administered nafamostat mesilate perioperatively.

Evaluations of Morbidity Including PVT

Morbidity was evaluated by Clavien's classification of surgical complications, and those with a score of Grade II or more were defined as positive [26]. Postoperative liver failure and bile leakage after liver surgery were evaluated according to the definitions of International Study Group of Liver Surgery [27,28].

At 5-7 days after hepatic resection, enhanced abdominal computed tomography (CT) was routinely performed for each patient to check for intra-abdominal problems such as an abscess around the resected stump or abnormality of hepatic blood flow. Postoperative PVT was evaluated using this enhanced abdominal CT [14].

Details of Postoperative Administration of Enoxaparin

The schedule of postoperative administration of enoxaparin is summarized in Fig. 1. To prevent hemorrhagic complications, subcutaneous injections of enoxaparin 20 mg were applied twice daily after the % PT had recovered to over 70%. Patients without an epidural catheter were given the 1st dose of enoxaparin within 24-36 hours after hepatic resection [6,7]. To prevent spinal epidural hematoma related to the decrease of anticoagulant proteins just after hepatic resection or the coexistence of liver cirrhosis, patients with epidural anesthesia were given their 1st dose of enoxaparin 12 hours after the removal of the epidural catheter. Twice-daily administration of enoxaparin was continued until discharge for at most 14 consecutive days [6,7,29].

Statistical Analysis

We compared the background characteristics, surgical outcomes, tumor-related factors, and short-term surgical results including symptomatic PE, symptomatic DVT, hemorrhagic complications, and postoperative PVT between the patients in the enoxaparin (+) and the enoxaparin (-) groups. Risk factors for postoperative PVT were analyzed in this series. Continuous variables are expressed as means $\pm$ SD and were compared using the Student's *t*-test. Categorical variables were compared using either the χ^2 test or Fisher's exact test, as appropriate. Variables at a P value of less than 0.15 on univariate analysis of risk factors for postoperative PVT were subjected to stepwise logistic regression analysis to identify the independent risk factors. All statistical analyses were performed with JMP® Pro 9.0.2 (SAS Institute Inc., Cary, NC). P-values less than 0.05 were considered significant.

Results

Comparisons of Patients' Background Characteristics, Surgical Outcomes, and Tumor-related Factors

The comparison of the patients' background characteristics, surgical outcomes, and tumor-related factors is shown in Table 1. The mean age (65 *vs.* 69 years; p = 0.01) and the mean Body Mass Index (BMI) (22.9 *vs.* 24.4; p < 0.01) were significantly higher in the enoxaparin (+) group. The ratio of females was higher in the enoxaparin (+) group

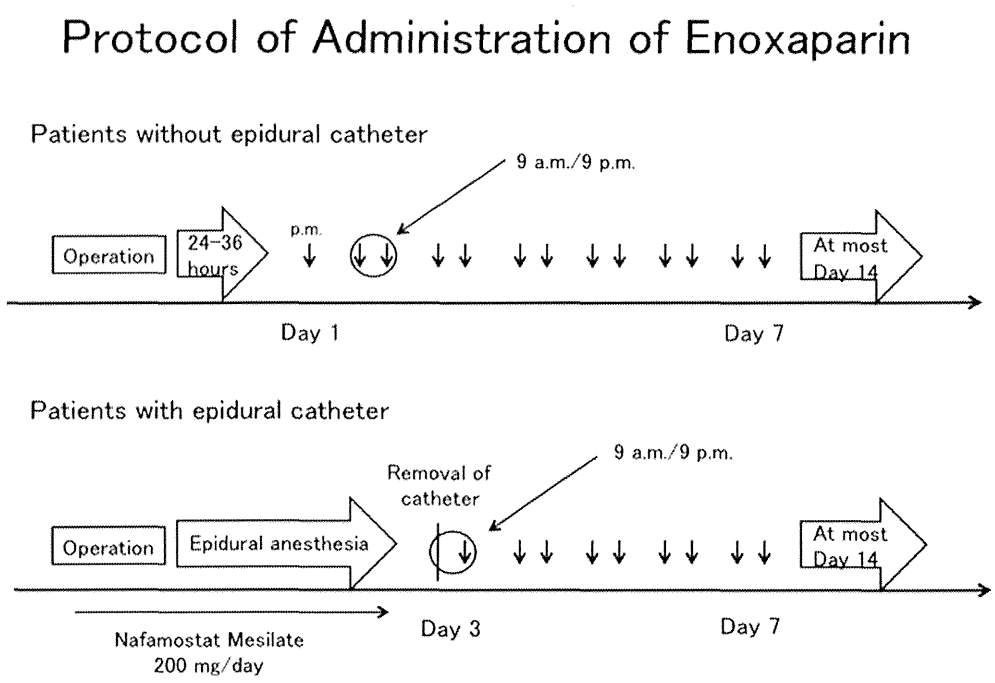

Fig. 1. The schedule of postoperative administration of enoxaparin (20 mg twice daily) is summarized. Patients without epidural catheter received their first dose of enoxaparin within 24-36 hours after hepatic resection. Patients with epidural anesthesia received their first dose of enoxaparin 12 hours after the removal of the epidural catheter. The administration of enoxaparin was continued until discharge for at most 14 consecutive days.

(13 *vs.* 40%), but the difference in ratio was not statistically significant ($p = 0.06$). There was no significant difference in any of the surgical outcomes or tumor-related factors between the two groups. The rates of the existence of histrogical cirrhosis were relatively high in both groups (34 *vs.* 23%; $p = 0.29$).

Comparisons of Short-term Surgical Outcomes

A comparison of short-term surgical outcomes is summarized in Table 2. There was no significant difference in mortality (0 *vs.* 0%; $p = 0.99$), entire morbidity rate (23 *vs.* 30%; $p = 0.29$), and the mean duration of the hospital stay (17 *vs.* 16 days; $p = 0.67$) between the two groups. Symptomatic PE occurred in one patient in the enoxaparin (-) group (0.4%), but the difference of complication rate was not statistically significant ($p = 0.63$). This patient was immediately treated with UFH, and the thrombus in the pulmonary artery disappeared 7 days after UFH treatment. This patient was discharged 25 days after operation with warfarin medication. No patient was complicated by symptomatic DVT in either group. Hemorrhagic complication occurred in one patient in the enoxaparin (-) group (0.4%), and surgical hemostasis with laparotomy was performed. Hemorrhagic complication also occurred in one patient in the enoxaparin (+) group (1.9%). This patient was complicated with a minor hemorrhage from the wound of the surgical drain, and this hemorrhage was immediately stopped by surgical suture under local anesthesia after discontinuance of enoxaparin. The difference in the rate of hemorrhagic complication was not statistically significant ($p = 0.79$). No patients were complicated with HIT in the enoxaparin (+) group.

The complication rate of PVT was significantly higher in the enoxaparin (-) group than that in the enoxaparin (+) group (10 *vs.* 2%; $p = 0.04$). Of course, preoperative abdominal CT confirmed that

Table 1
Background characteristics, surgical outcomes, tumor-related factors.

Variables	Enoxaparin (-) (n = 228)	Enoxaparin (+) (n = 53)	p-Value
Back ground characteristics			
Age	65 ± 12	69 ± 10	0.01
Male/Female	169/29	32/21	0.06
BMI	22.9 ± 3.1	24.4 ± 4	<0.01
DM (%)	47(21%)	9 (17%)	0.37
Total bilirubin (mg/dL)	0.8 ± 0.4	0.8 ± 0.3	0.90
Albumin (%)	4.0 ± 0.5	4.0 ± 0.5	0.94
ICG R15 (%)	13.9 ± 8.9	13.4 ± 7.8	0.72
Child-Pugh; A/B	227/1	53/0	0.27
Liver Damage: A/B	176/52	42:11	0.81
Pit (x 10^4//il)	16.4 ± 6.5	16.7 ± 5.1	0.69
Histological cirrhosis: yes/no	77/151	12/41	0.29
Surgical outcomes			
Surgical time (min)	350 ± 146	343 ± 151	0.74
Surgical blood loss (g)	576 ± 645	593 ± 800	0.87
Transfusion (%)	31 (14%)	4 (8%)	0.23
Major hepatectmy : yes/no	41/181	10/43	0.83
Liver ischemic time (mm)	46 ± 44	41 ± 33	0.45
Laparoscopic procedure (%)	49 (21%)	13 (25%)	0.63
Tumor-related factors			
Tumor size (cm)	3.5 ± 3.1	3.3 ± 2.3	0.73
Number of tumors	1.9 ± 1.5	1.6 ± 1.6	0.35
stage III or IV (%)	95 (45%)	25 (50%)	0.54

Abbreviations:
DM; Diabetis Melitus, ICG R15: indocyanine green retention rate at 15 minutes,

Table 2
Short-term surgical results.

Variables	Enoxaparin (-) (n = 228)	Enoxaparin (+) (n = 53)	p-value
Mortality (%)	0 (0%)	0 (0%)	0.99
Morbidity* (%)	53 (23%)	16 (30%)	0.29
Symptomatic PE (%)	1 (0.4%)	0 (0%)	0.63
Symptomatic DVT (%)	0 (0%)	0 (0%)	0.99
Hemorrhagic complication (%)	1 (0.4%)	1 (1.9%)	0.79
Bile leakage* (%)	8 (4%)	5 (10%)	0.08
Posthepatectomy liver failure* (%)	50 (22%)	8 (16%)	0.31
PVT (%)	23 (10%)	1 (2%)	0.04
Duration of hospital stay (days)	17 ± 15	16 ± 16	0.67

Abbreviations: PE: pulmonary embolism. DVT: deep vein thrombosis. PVT: portal vein thrombosis.
* Morbidity: Clavien-Dindo Grade II or more.
* Bile leakage and posthepatectomy liver failure: Defined by International Study Group of Liver Surgery.

there was no PVT in all patients. The typical cases of postoperative PVT are shown in Fig. 2. In the case shown in Fig. 2A, a partial thrombus of portal vein at the umbilical portion was found after anterior segmentectomy for HCC without the administration of enoxaparin. In the case shown in Fig. 2B, a partial thrombus of the portal vein at the main trunk was found after right lobectomy for HCC without the administration of enoxaparin. Both patients were immediately treated by UFH, and the medication was changed to warfarin after the confirming by enhanced abdominal CT that the PVT had not propagated around 7-14 days after treatment. The PVT disappeared in both patients at enhanced abdominal CT 3 months after operation, at which point warfarin administration was stopped.

Risk Factors for Postoperative PVT

A comparison between the PVT (-) group (n = 257) and the PVT (+) group (n = 24) in terms of patients' background, surgical outcomes, tumor-related factors, and other factors is summarized in Table 3. There were no significant differences in patients' background characteristics between the two groups. The mean age was slightly higher in the PVT (+) group than that in the PVT (-) group (66 *vs.* 67 years), but not significantly (p = 0.16). Concerning the surgical outcomes, the mean surgical time was significantly longer in the PVT (+) group than in the PVT (-) group (342 *vs.* 421 minutes; p = 0.01). The mean liver ischemic time was longer in the PVT (+) group than in the PVT (-) group, but not significantly (43 *vs.* 65 minutes; p = 0.06). Concerning tumor-related factors, HCC-positive rate was significantly higher in the PVT (+) group than in the PVT (-) group (62 *vs.* 88%; p = 0.04). The ratio of patients receiving enoxaparin was significantly lower in the PVT (+) group than in the PVT (-) group (20 *vs.* 4%; p = 0.03).

Independent Risk Factor for Postoperative PVT

The results of the stepwise logistic regression analysis are summarized in Table 4. Surgical time ≥ 360 minutes (odds ratio 6.66, p < 0.01) and non-treatment with enoxaparin (odds ratio 2.49, p = 0.03) were revealed to be independent risk factors for postoperative PVT in our series.

Table 3
Risk factors for postoperative PVT.

Variables	PVT (-) (n = 257)	PVT (+) (n = 24)	p-Value
Back ground characteristics			
Age	66 ± 12	67 ± 11	0.16
Male/Female	185/72	16/8	0.45
BMI	23.2 ± 3.3	23.2 ± 3.6	0.53
DM (%)	53(21%)	3 (13%)	0.37
Total bilirubin (mg/dL)	0.8 ± 0.4	0.7 ± 0.3	0.40
Albumin (%)	4.0 ± 0.5	4.0 ± 0.6	0.88
ICG R15 (%)	13.9 ± 8.8	13.8 ± 8.3	0.64
Child-Pugh; A/B	256/1	24/0	0.88
Liver Damage: A/B	197/60	20/4	0.48
Pit (x 10^4//il)	16.4 ± 6.3	17.1 ± 5.3	0.52
Histological cirrhosis: yes/no	78/179	11/13	0.21
Surgical outcomes			
Surgical time (min)	342 ± 146	421 ± 137	0.01
Surgical blood loss (g)	581 ± 693	599 ± 484	0.32
Transfusion (%)	29(11%)	6 (25%)	0.16
Major hepatectmy : yes/no	45/212	6/18	0.46
Liver ischemic time (mm)	43 ± 41	65 ± 56	0.06
Laparoscopic procedure (%)	59 (23%)	3 (13%)	0.32
Tumor-related factors			
Tumor size (cm)	3.5 ± 3.0	3.7 ± 2.5	0.20
Number of tumors	1.9 ± 1.6	1.6 ± 1.0	0.40
HCC (+) (%)	160 (62%)	21 (88%)	0.04
Other factor			
Enoxaparin (+) (%)	52 (20%)	1 (4%)	0.03

Abbreviations:
PVT; Portal vein thrombosis. DM: Diabetis Melitus, ICG R15; indocyanine green retention rate at 15 minutes.

Discussion

With regard to VTE chemoprophylaxis, surgeons have always needed to balance the risk of peri-operative bleeding complications against the risk of VTE. Liver surgeons have historically withheld perioperative VTE chemoprophylaxis mainly as a result of the perceived risk of perioperative bleeding, with the hypothesis that transient liver dysfunction after a hepatic resection produces anticoagulation effects. We previously reported that recent advances in surgical techniques in liver surgery accounted for a decrease in the peri-operative bleeding complication rate to as low as 1% [30]. Tzeng CW et al reported that 30-day VTE occurred 163 of 5651 patients (2.9%) with hepatic resection, and was strongly associated with mortality [31]. Therefore, recent years

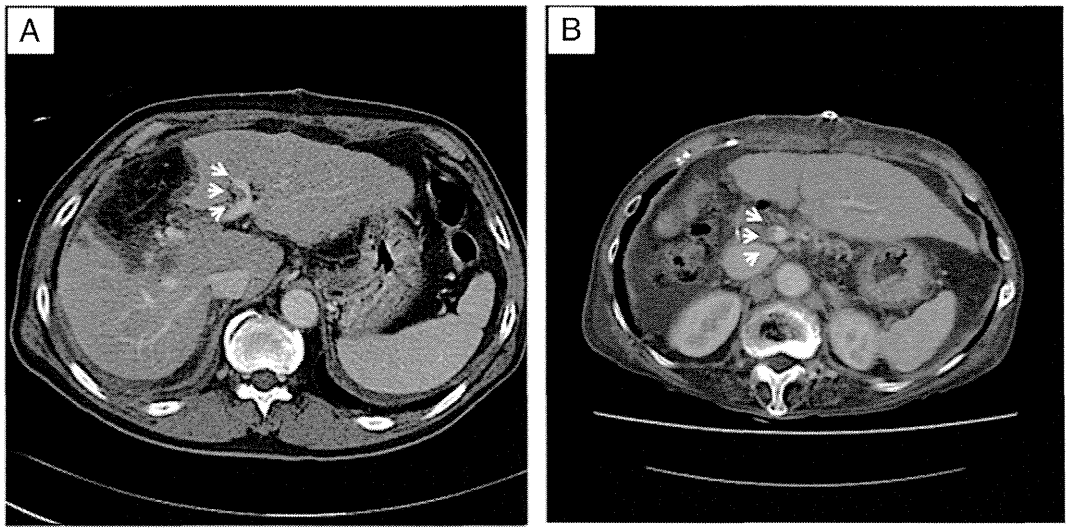

Fig. 2. Typical cases of postoperative PVT after hepatic resection. In Fig. 2A, a partial thrombus of portal vein at the umbilical portion was found after anterior segmentectomy for HCC without the administration of enoxaparin. In Fig. 2B, a partial thrombus of the portal vein at the main trunk was found after right lobectomy for HCC without the administration of enoxaparin.

Table 4
Independent risk factors for PVT.

Variables	Coefficient/SE	Odds ratio	p-value
Surgical time ≥ 360 min.	2.58	6.66	<0.01
Enoxaparin (-)	1.58	2.49	0.03
HCC (+)	1.32	1.68	0.26
Liver ischemic time ≥ 45 min.	0.73	1.35	0.57

have seen a keen interest in chemoprophylaxis as a possible method of reducing postoperative VTE without increasing the risk of bleeding complications in liver surgery.

According to the clinical results of efficacies and risks in Japanese patients (6.7), enoxaparin 20 mg twice daily is the standard regimen for VTE chemoprophylaxis after surgery under the national health insurance in Japan. We applied this regimen for patients who underwent hepatic resections as described in Fig. 1. To minimize the risk of spinal epidural hematoma, we did not concomitantly administrate enoxaparin to patients with epidural anesthesia. VTE and PVT formation would start during operation, however, it is important to start anticoagulant drugs as early as possible. According to the recommendation of Rosencher et al, the removal of the epidural catheter should be over "X + 2Y" hours (X; Tmax, Y; t1/2) after the last application of anticoagulant drugs, and the next application of anticoagulant drugs should be over "8-Tmax" hours later [32]. Concerning the enoxaparin, Tmax is 2.3 hours, t1/2 is 3.2 hours, X + 2Y = 8.7 hours, and 8-X = 5.7 hours [33]. This recommendation should be useful to enable earlier administration of enoxaparin during epidural anesthesia in patients who undergo hepatic resections.

To minimize the risk of bleeding complication, we administered enoxaparin to patients with %PT ≥ 70%. The prolongation of PT demonstrates the decrease of procoagulant proteins, however, cirrhotic patients with low %PT sometimes develop arterial, portal or venous thrombosis which is partly attributed to hypercoagulation [34]. In addition, increasing clinical evidence suggests that the prolonged PT should not be a reason to withhold anticoagulation in patients with cirrhosis or after hepatic resection [35–40]. Despite a postoperative prolongation of the PT, a thrombotic risk in patients after hepatic resection would exist because of a concomitant decline in pro- and anticoagulants which is not reflected in the PT which only assesses pro-coagulant proteins. Therefore, our patients' exclusion criteria of %PT ≤ 70% should be examined further in a detailed clinical study.

Ejaz A et al reported that the preventative effect of UFH for postoperative VTE in hepatic resections was negative in both 90-day DVT (2.1 *vs.* 3.7%; p = 0.33) and 90-day PE (2.1 *vs.* 1,8%; p = 0.81) [41]. Also LMWH (nadroparin or enoxaparin) was previously observed to have no significant preventative effects against postoperative VTE in patients who underwent resections of HCC developed from cirrhosis (1.4 *vs.* 0.6%; p = 0.53) [42]. In our series, the preventative effect of enoxaparin for postoperative symptomatic PE and DVT after hepatic resection was not significant. However, the complication rate of PVT after hepatic resection was significantly decreased by enoxaparin administration (10 *vs.* 2%; p = 0.04).

Postoperative PVT is a potentially life-threatening complication that occurs after hepatobiliary pancreatic surgery, especially in liver transplantation or pancreaticoduodenectomy [11]. In our series, postoperative PVT as evaluated by enhanced abdominal CT on postoperative day 5-7 was evident in 24 patients (8.5%). This is the first report concerning the preventative effects of anticoagulant drugs for PVT after hepatic resection. Theoretically, PVT cannot be prevented by mechanical prophylaxis by elastic compression leg stocking and IPC; therefore, the chemical prophylaxis for PVT is essential. PVT after hepatic resection especially in cirrhotic patients would lead to postoperative liver failure [14], intractable ascites, or gastrointestinal variceal hemorrhage; therefore, the prevention and early diagnosis of, and rapid initiation of treatment for PVT is very important in patients undergoing hepatic resection.

In our series, the independent risk factors for PVT after hepatic resection were surgical time ≥ 360 minutes (odds ratio 6.66, p < 0.01) and non-treatment with enoxaparin (odds ratio 2.49, p = 0.03). The possible causes for the formation of postoperative PVT are stasis of blood flow in portal vein, a hypercoaguable state, and endothelial injury. The significantly longer surgical time in PVT (+) patients would relate to longer liver ischemic time, longer venous stasis time caused by mobilization of liver, and longer and more frequent tractions of the portal vein. These procedures cause stasis of blood flow in the portal vein or endothelial injury of the portal vein. Patients with HCC and cirrhosis are more likely to be in a hypercoagulable state compared to those with other disease entities. Several reports have already mentioned the safety and efficacy of LMWH including enoxaparin for PVT in patients with advanced cirrhosis [43–45], and our new finding of the preventive effect of enoxaparin for postoperative PVT after hepatic resection would support the usefulness of enoxaparin for treatment against PVT.

In conclusion, postoperative anticoagulant therapy with enoxaparin 20 mg twice daily could prevent postoperative PVT in patients undergoing hepatic resection for liver cancers without increase of bleeding complication. The inclusion/exclusion criteria, and the protocol of administering enoxaparin, especially for patients with epidural anesthesia, should be further investigated to achieve the best efficacy of enoxaparin as a prophylactic measure against postoperative VTE in patients who have undergone hepatic resection.

Conflict of Interest

We declare that we have no conflict of interest to disclose.

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Hepatology Research

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Original Article

Skeletal muscle area correlates with body surface area in healthy adults

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Aim: Depletion of skeletal muscle mass (sarcopenia) predicts survival in patients with cancer or liver cirrhosis. Recently, many reports have used computed tomography (CT) to measure muscle area to define sarcopenia. However, the definition of sarcopenia using CT has not been fully determined. The aim of this study was to establish formulae to calculate the standard area of skeletal muscle.

Methods: Forty-five healthy adults (24 men and 21 women, aged 21–66 years) who wished to donate part of their liver for transplantation underwent CT. Cross-sectional areas (cm^2) of skeletal muscle were measured at the caudal end of the third lumbar vertebra. Regression analysis was performed to establish formulae to calculate the standard area of skeletal muscle. A validation conducted on 30 other healthy adults was performed to check the accuracy of formulae.

Results: Men had a median skeletal muscle area of 155.0 cm^2 (range, 114.0–203.0), compared with 111.7 cm^2 (range, 89.8–139.3) in women ($P < 0.001$). Furthermore, skeletal muscle area significantly correlated with body surface area (BSA) in men ($P < 0.0001$, $r^2 = 0.60$) and women ($P < 0.0001$, $r^2 = 0.78$). The formulae to calculate skeletal muscle area were $126.9 \times BSA - 66.2$ in men and $125.6 \times BSA - 81.1$ in women. The estimated muscle area significantly correlated with actual muscle area in men ($P = 0.003$, $r^2 = 0.64$) and women ($P = 0.0001$, $r^2 = 0.70$).

Conclusion: Sarcopenia can be defined by the difference between measured data and calculated data using our new formulae.

Key words: body surface area, computed tomography, sarcopenia, skeletal muscle area

INTRODUCTION

THE LOSS OF skeletal muscle mass in advanced cancer patients is the result of an imbalance between protein synthesis and degradation.[1] Depletion of skeletal muscle mass (sarcopenia) can predict survival in patients with various kinds of cancer[2–4] or patients with liver cirrhosis.[5] Up to 50% of patients with advanced cancer have frank sarcopenia.[1] Cachexia has long been recognized as an adverse effect of cancer.[6] Recently, Fearon *et al.* reported a definition of cachexia,[6] which included weight loss of more than 5% over the past 6 months, or a body mass index (BMI) of less than 20 and any degree of weight loss of more than 2%, or appendicular skeletal muscle index consistent with sarcopenia and any degree of weight loss of more than 2%.

Van Vledder *et al.*[2] performed computed tomography (CT) based on a simple measurement of skeletal muscle area, which was normalized for stature using BMI. They defined sarcopenia as skeletal muscle area of less than 41.1 cm^2/m^2 for women and less than 43.75 cm^2/m^2 for men, and reported that 20% of patients studied were sarcopenic. The psoas muscle, measured by CT, was also used to predict patient survival rate after liver transplantation.[7] This study revealed that the risk of mortality increased as psoas muscle area decreased (hazard ratio = 3.7 per 10 cm^2 decrease in psoas muscle area),

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Conflict of interest: The authors declare no conflict of interest.

Author contribution: Tomoharu Yoshizumi designed and performed the study, collected and analyzed data, and wrote the paper; Ken Shirabe, Toru Ikegami, Norifumi Harimoto, Hidekazu Nakagawara and Yuji Soejima performed the study; Yo-ichi Yamashita and Tetsuo Ikeda collected the data; Takeo Toshima analyzed the data; Yoshihiko Maehara provided critical comment.

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but the authors did not define sarcopenia in their study. Thus, the definition of sarcopenia using CT measurement has been set using an unfounded cut-off level, and has not been accurately determined as yet.

In this study, we retrospectively studied healthy Japanese adults to establish formulae to calculate standard muscle area to enable an easy and accurate definition of sarcopenia.

METHODS

Control subjects

DATA ON 45 healthy adults (24 male; 21 female; aged 21–66 years old) who hoped to be liver donors were reviewed. Donor candidates who performed resistance muscle training and hard labor were excluded from this study, because these conditions affected skeletal muscle mass. These conditions should be in exclusion criteria of subjects. Forty of 45 candidates were eligible to be donors and underwent left or right hepatectomy after several studies including 2-mm slice abdominal CT. These CT images were used for determining the quantity of skeletal muscle and the area of the psoas muscle.[2,6] Cross-sectional areas (cm^2) of skeletal muscle or the psoas muscle were manually measured at the caudal end of the third lumbar vertebra (Fig. 1).[2,6]

Bodyweight (BW) and height recorded on the donor charts were used for calculating body surface area (BSA) and BMI. Equations for BSA[8] and BMI[9] were as follows:

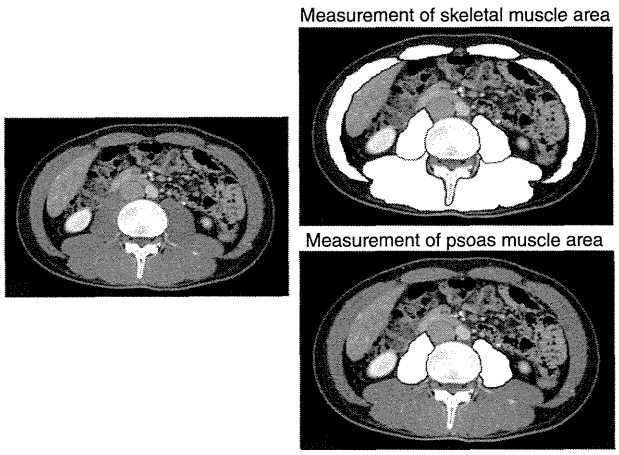

Figure 1 Representative computed tomography for measurement of the area of the skeletal muscle and psoas muscle. Cross-sectional areas (cm^2) of skeletal muscle (upper right) or the psoas muscle (lower right) were manually measured at the caudal end of the third lumbar vertebra.

$$\text{BSA}\,(m^2) = \text{square root}\,(\text{BW}\,[kg] \times \text{height}\,[cm]/3600)$$

$$\text{BMI}\,(kg/m^2) = \text{BW}\,(kg)/\text{height}\,(m)^2$$

Our goal was to develop a simple formula for men and women relating a single factor to the skeletal or psoas muscle area. Measured skeletal or psoas muscle area was plotted against age, height, BW, BSA or BMI, and a formula was developed using simple regression. The significance of the regressions was determined as previously described.[10]

Validation set

We then applied our formula to estimate skeletal muscle area in another 30 healthy donors (15 male; 15 female; aged 20–58 years). The significance of regressions between estimated skeletal muscle area by our formula and measured skeletal muscle area was determined as previously described.[10]

A *P*-value of less than 0.05 was considered statistically significant. Data are expressed as mean ± standard deviation (SD). All statistical analyses were performed using StatView ver. 5.0 software (SAS Institute, Cary, NC, USA).

The study protocol was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the institutional review board.

RESULTS

Control set

AGE, BODY SIZE parameters and muscle area of the donors are shown in Table 1. Except for BMI, body size parameters were significantly greater in men compared with women ($P < 0.001$, Table 1).

The measured skeletal muscle area was significantly correlated with BW ($r^2 = 0.76$, $P < 0.001$), height ($r^2 = 0.61$, $P < 0.001$), BMI ($r^2 = 0.28$, $P = 0.0002$) and BSA ($r^2 = 0.79$, $P < 0.001$) (Fig. 2). The regression analysis demonstrated that measured skeletal muscle area was correlated highest with BSA among the four parameters. On the basis of these results, the following equations were derived from our data by linear regression analysis to predict the skeletal muscle area for normal adults (Fig. 3):

$$\text{Skeletal muscle area}\,(cm^2)\,\text{for males} = 126.9 \times \text{BSA} - 66.2$$

$$\text{Skeletal muscle area}\,(cm^2)\,\text{for females} = 125.6 \times \text{BSA} - 81.1$$

Table 1 Body size and muscle mass in 45 healthy adults

	Sex	
	Male	Female
n	24	21
Age (years) (range)	43.4 ± 14.7 (21–66)	40.7 ± 10.0 (24–63)
Body height (cm) (range)	170.2 ± 6.9* (155.0–180.0)	158.7 ± 5.3* (146.0–167.0)
Bodyweight (kg) (range)	64.4 ± 8.6* (46.0–82.1)	53.6 ± 5.6* (46.0–65.0)
Body surface area (m^2) (range)	1.74 ± 0.15* (1.43–2.01)	1.54 ± 0.09* (1.38–1.73)
Body mass index (range)	22.2 ± 2.0 (18.0–26.2)	21.3 ± 2.2 (18.0–25.3)
Skeletal muscle area (cm^2) (range)	155.0 ± 23.9* (114–203)	111.7 ± 13.0* (89.8–139)
Psoas muscle area (cm^2)† (range)	20.6 ± 8.8* (13.9–30.4)	11.1 ± 2.7* (7.3–16.4)

*$P < 0.01$.
†Five data (four in males and one in females) were missing.

Figure 4 shows the relationship between the measured psoas muscle area and four body size parameters. The relationship was statistically significant, but the value of the coefficient of determination, "r^2," was 0.41 with BSA, 0.39 with BW, 0.33 with height and 0.15 with BMI.

Figure 5 shows the relationship between age and measured skeletal muscle area or measured psoas muscle area. Skeletal muscle area or psoas muscle area was not significantly related to age ($r^2 = 0.04$, $P = 0.22$ in skeletal muscle area; $r^2 = 0.07$, $P = 0.11$ in psoas muscle area).

Validation set

Figure 6 shows the relationship between measured skeletal muscle area and estimated skeletal muscle area calculated using the sex-specific formula using 30 healthy adults (15 male and 15 female). The estimated muscle area significantly correlated with actual muscle area in men ($P = 0.003$, $r^2 = 0.64$) and women ($P = 0.0001$, $r^2 = 0.70$). The mean difference between the calculated muscle area and measured area was −1.94 ± 12.8 cm^2 (−22.8 to 18.7) for males and 9.7 ± 7.0 cm^2 (−2.5 to 21.3) for females.

Representative cases

Figure 7 shows two representative cases of sarcopenia, who underwent living donor liver transplantation (LDLT) because of decompensated liver cirrhosis. Case 1 was a 51-year-old male. His physical parameters were as follows: height, 170 cm; weight, 54 kg; BSA, 1.6 m^2; and

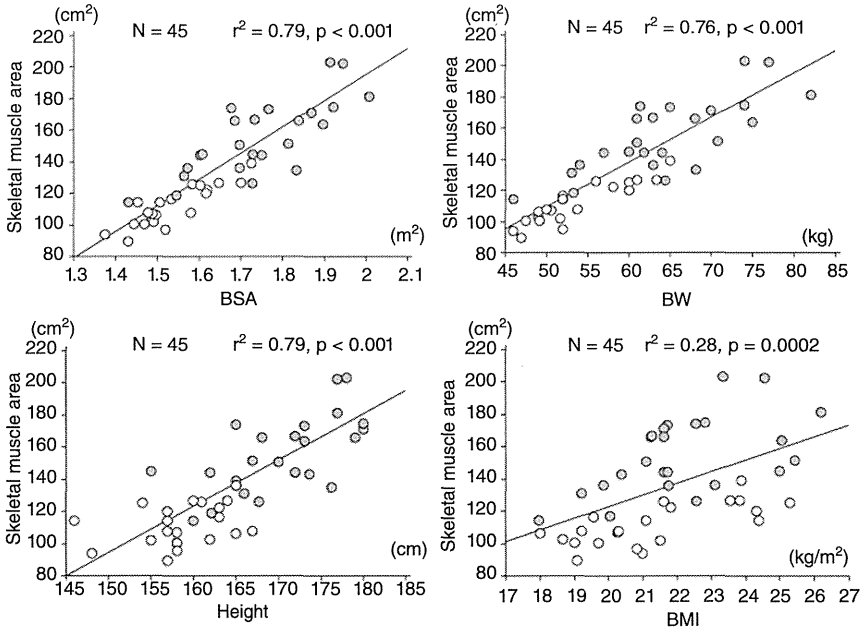

Figure 2 Relationship between each body size parameter and measured skeletal muscle in 45 healthy adults. Measured skeletal muscle mass was significantly correlated with body surface area (BSA) (upper left, $r^2 = 0.83$, $P < 0.001$), bodyweight (BW) (upper right, $r^2 = 0.79$, $P < 0.001$), body height (lower left, $r^2 = 0.66$, $P < 0.001$) or body mass index (BMI) (lower right, $r^2 = 0.28$, $P = 0.004$). Skeletal muscle mass was correlated highest with BSA among the four parameters. Closed circles for males, open circles for females.

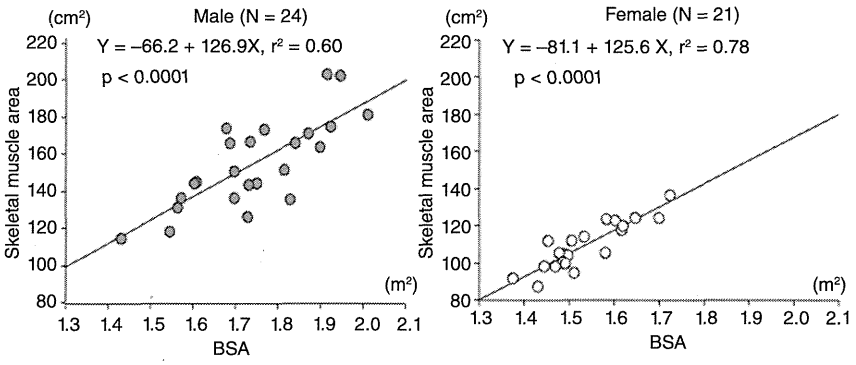

Figure 3 Relationship between body surface area (BSA) and measured skeletal muscle mass in each sex. Closed circles for males, open circles for females. The skeletal muscle mass in each sex was predicted using the following equations: skeletal muscle mass (cm^2) for males (left) = 126.9 × BSA – 66.2; and skeletal muscle mass (cm^2) for females (right) = 125.6 × BSA – 81.1.

his skeletal muscle area, measured using preoperative CT, was 69.3 cm^2 (Fig. 7a). The skeletal muscle area calculated by our formula was 139.6 cm^2. The difference between the calculated mass and measured mass was 70.3 cm^2. Thus, we retrospectively diagnosed that he had sarcopenia before LDLT. His Model for End-Stage Liver Disease (MELD) score was 24 points before LDLT, and he received an extended left and caudate lobe graft weighing 345 g (30.1% of standard liver weight) from his 28-year-old son. Although the graft functioned well after reperfusion, he died 114 days after LDLT because of bacterial sepsis. Case 2 was a 56-year-old female. Her physical parameters were as follows: height, 150 cm; weight, 53 kg; BSA, 1.49 m^2; and her skeletal muscle area was 63.1 cm^2 (Fig. 7b). Skeletal muscle area calculated by our formula was 105.5 cm^2. The difference between the calculated mass and measured mass was 42.4 cm^2. Her MELD score was 25 points before LDLT, and she received an extended left and caudate lobe graft weighing 426 g (41.0% of standard liver weight) from her 51-year-old brother. Although she had bacterial sepsis and thrombotic microangiopathy after LDLT, she recovered and was discharged from hospital 53 days after LDLT.

DISCUSSION

IN THIS STUDY, we found that skeletal muscle area measured by CT was highly correlated with BSA. In addition, measurement of skeletal muscle area was

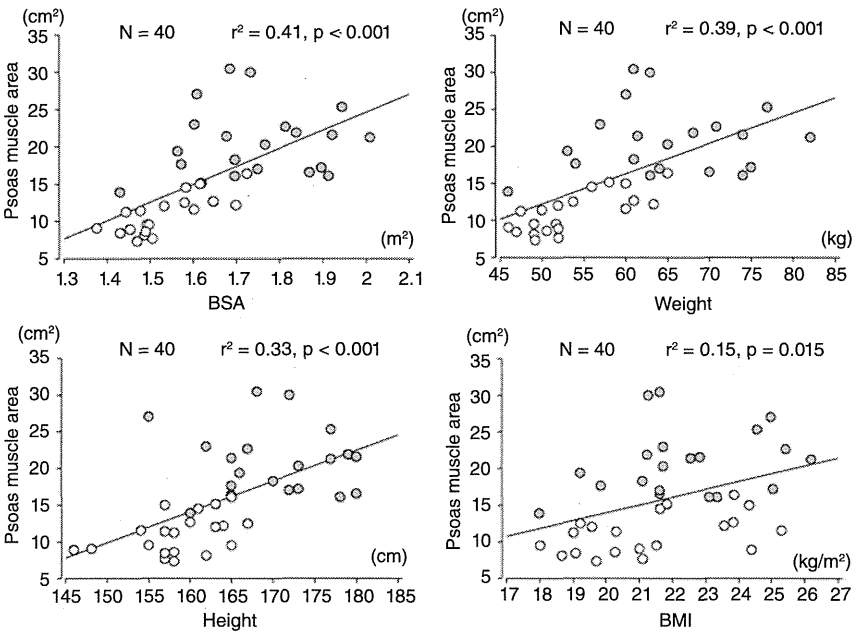

Figure 4 Relationship between each body size parameter and measured psoas muscle area in 40 healthy adults. The measured psoas muscle area was correlated with body surface area (BSA) (upper left, $r^2 = 0.41$, $P < 0.001$), bodyweight (upper right, $r^2 = 0.39$, $P < 0.001$), body height (lower left, $r^2 = 0.33$, $P < 0.001$) or body mass index (BMI) (lower right, $r^2 = 0.15$, $P = 0.015$). Closed circle for males, opened circle for females.