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How to cite

KAMBAKAMBA, Patryk et al. Early Postoperative Serum Phosphate Drop Predicts Sufficient Hypertrophy after Liver Surgery. In: Annals of surgery, 2023, vol. 278, n° 5, p. 763–771. doi: 10.1097/SLA.0000000000006013

This publication URL: https://archive-ouverte.unige.ch/unige:176150

Publication DOI: 10.1097/SLA.0000000000006013

Annals of Surgery Publish Ahead of Print
DOI:10.1097/SLA.0000000000006013

Early Postoperative Serum Phosphate Drop Predicts Sufficient Hypertrophy after Liver Surgery

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PK and MAS share first authorship, DB and HP share senior authorship.

Funding: None

Author contribution: All authors contributed significantly according to the journal guideline

Category: Original article

Key words: ALPPS, liver regeneration, serum phosphate, phosphorus, hypophosphatemia

Short running head: Hyperphosphatemia and liver regeneration

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STRUCTURED ABSTRACT

Objective: The aim of this study was to assess the impact of postoperative hypophosphatemia on liver regeneration after major liver surgery in the scenario of ALPPS (Associating Liver Partition with Portal vein ligation for Staged hepatectomy) and living liver donation (LLD).

Background: Hypophosphatemia has been described to reflect the metabolic demands of regenerating hepatocytes. Both, ALPPS and LLD, are characterized by an exceptionally strong liver regeneration and may be of particular interest in the context of posthepatectomy hypophosphatemia.

Methods: Serum phosphate changes within the first 7 postoperative days after ALPPS (n=61) and LLD (n=54) were prospectively assessed and correlated with standardized volumetry after one week. In a translational approach, postoperative phosphate changes were investigated in mice and *in vitro*.

Results: After ALPPS stage-1 and LLD, serum phosphate levels significantly dropped from a preoperative median of 1.08 mmol/L (IQR 0.92-1.23) and 1.07 mmol/L (IQR 0.91-1.21) to a postoperative median nadir of 0.68 mmol/L and 0.52 mmol/L, respectively. A pronounced phosphate drop correlated well with increased liver hypertrophy (p<0.001). Patients with a low drop of phosphate showed a higher incidence of posthepatectomy liver failure after ALPPS (7 vs. 31%, p=0.041). Like in human, phosphate drop correlated significantly with degree of hypertrophy in murine ALPPS and hepatectomy models (p<0.001). Blocking phosphate transporter (Slc20a1) inhibited cellular phosphate uptake and hepatocyte proliferation *in vitro.*

Conclusion: Phosphate drop after hepatectomy is a direct surrogate marker for liver hypertrophy. Perioperative implementation of serum phosphate analysis has the potential to detect patients with insufficient regenerative capacity at an early stage.

ABBREVIATIONS

ALPPS	Associating Liver Partition with Portal vein ligation for Staged hepatectomy
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
BW	Body weight
DH	Degree of liver hypertrophy
DNA	Deoxyribonucleic acid
FACS	Fluorescence activated cell sorting
FLR	Future liver remnant
HCC	Hepatocellular carcinoma
H&E	Hämatoxylin-eosin
IQR	Interquartile range
ISGLS	International Study Group of Liver Surgery
KGR	Kinetic growth rate
LLD	Living liver donation
p-ALPPS	Partial ALPPS
PFA	Phosphonoformic acid
PHC	Perihilar cholangiocarcinoma
PHLF	Post-hepatectomy liver failure
POD	Postoperative day
PTH	Parathyroid hormone
PVL	Portal vein ligation
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
sFLR	Standardized future liver remnant
SD	Standard deviation
Slc20A1	Solute carrier family 20 member 1

INTRODUCTION

The liver has the unique potential to regenerate after major tissue loss.[1, 2] The process of liver regeneration demands tremendous amounts of phosphate for the synthesis of nucleotidetriphosphates during DNA replication. In this context, the serum phosphate drop has been described to reflect the metabolic expenditure of regenerating hepatocytes after major liver resection.[3-6] The regenerative impulse is set immediately after resection and physiologically reaches its mitotic climax within the first 72 postoperative hours.[2, 7]

Theoretically, hypophosphatemia may be of particular interest in surgical procedures inducing a pronounced regenerative signal. In that context, the so called "Associated Liver Partition and Portal vein ligation for Staged hepatectomy" (ALPPS) has gained attention, due to enhanced liver regeneration.[8, 9] Basically ALPPS is a two-stage hepatectomy which combines portal vein ligation with parenchymal transection and induces a strong regenerative impulse enabaling extended resections already 1 week after ALPPS stage-1.[10] Whereas the regenerative boost after ALPPS was initially considered unprecedented, liver resections in healthy livers for living liver donation (LLD) showed comparable growth kinetics of the liver remnant. [4, 8, 9, 11] This accelerated liver hypertrophy renders both, ALPPS and LLD, a unique setting for studies in the field of liver regeneration.

To date, hypophosphatemia and direct volume gain after major liver surgery has not been analysed. Therefore, the aim of this study was, to assess postoperative phosphate changes as an early surrogate marker for liver hypertrophy, in patients undergoing two major liver procedures, namely ALPPS and LLD, with the most pronounced regenerative response. Additionally, mechanisms of posthepatectomy hyposphosphatemia were investigated in mice and *in vitro*.

METHODS

Study design

All patients undergoing ALPPS at the University Hospital Zurich from January 2011 to December 2018 were included. LLD was performed at the Transplant Unit of the University Hospital Ankara between May 2018 and May 2019. Data were collected in a prospective database using standardized forms approved by the independent ethics committee of Zurich (KEK-ZH-Nr. 2018-02391) and Ankara.

ALPPS and living liver donor surgery

ALPPS procedure (complete and partial) was performed as previously described.[8, 9 12] All living liver donors donated the right lobe.[13]

Assessment of morbidity and liver failure

Surgical complications were ranked according to the Dindo-Clavien classification.[14, 15] Major complications were defined as ≥3b. Liver failure was defined according to the "50-50" and the ISGLS criteria.[16, 17]

Volumetric measurements

All ALPPS patients and LLDs had preoperative imaging and standardized imaging at day 7 after surgery. A commercially available software (Myrian®, version 1.14 Intrasense; Paris, France) was used. Volumetric measurements followed institutional standards and were previously described.[18, 19] Additionally, dynamic volumetric parameters such as degree of hypertrophy (DH) and kinetic growth rate (KGR) were routinely calculated. [4, 11, 20, 21]

Clinical serum phosphate levels and kidney function

Serum phosphate levels [mmol/L] and kidney function (creatinine [µmol/L] and glomerular filtration rate [mL/min]) were measured daily, starting one day prior to surgery until postoperative day 7 after surgery. Following phosphate parameters were assessed: the lowest phosphate value (nadir), the day of phosphate nadir, the fastest phosphate drop per day as compared to preoperative baseline [%/d], and the day of fastest phosphate drop. The cut-off for low and high phosphate drop per day was set at the median.

Phosphate replacement policies

All living donor patients had no phosphate replacement treatment after hepatectomy while some patients of the ALPPS cohort with pronounced hypophosphatemia had phosphate substitution. Replacement was only considered for ALPPS patients with moderate (0.3-0.59 mmol/L) or severe hypophosphatemia (>0.3 mmol/L). Whenever phosphate substitution in ALPPS patients was administered, it was always done in a reactive manner after serum phosphate drop occurred. Oral substitution (Phosphate Sandoz®) was the standard treatment and intravenous replacement was restricted to patients incapable of oral intake.

Experimental ALPPS and hepatectomy rodent model

All animal experiments were performed in accordance with Swiss Federal Animal Regulations (*#26179/ZH081/15*). Male C57BL/6 mice (Envigo, Horst, Netherlands) aged 8-12 weeks were used. Six mice per experimental group were analysed. Sham laparotomy, 90% portal vein ligation without transection of liver parenchyma (PVL), 25% partial ALPPS (p-ALPPS), 80% p-ALPPS and different extents of hepatectomies (50%, 68%, and 86%) were performed, as previously described.[22, 23] [24, 25] The 86%-hepatectomy is characterized by impaired regeneration and produces features consistent with small-for-size-syndrome. [24, 26]

Experimental assessment of liver regeneration, serum phosphate level, and hepatic

DNA content

Animals were sacrificed 48 hours after surgery. Liver regeneration was assessed by liver/body weight ratio and immunostaining for proliferative markers Ki-67 (Abcam, ab16667, Cambridge, United Kingdom) and pH3 (Millipore, 06-570-3KL, Billerica, USA). Serum phosphate levels in mice were measured with a calorimetric phosphate assay (ab65622 abcam) after 30 µL tail vein blood were withdrawn from each animal prior to surgery, at 24 hours after surgery, and before harvest. Liver DNA content was measured by spectrophotometric quantification using a Nanodrop One (ThermoFischer Scientific, Waltham, USA).

***In-vitro* experiments**

Immortalized murine hepatocellular TIB-75 cells were cultured in various concentrations of phosphate (0, 10, 100, 1000 µM). Phosphate transport blocker phosphonoformic acid (PFA, 1283302, Sigma Aldrich) was used to inhibit cellular phosphate uptake.[27] Supernatant was frozen at indicated time points and phosphate concentrations were measured as described above. Proliferation was measured by staining with live-dead staining (423105, Biolegend, San Diego, CA) and consequent counting of vital cells on a BD-FACS-Canto II.

Phosphate transporters in murine and human liver samples

Transcription of the sodium-dependent phosphate transporter 1 Slc20a1 (Hs00965587_m1) and Slc20a2 (Hs00198840_m1) was assessed in large scale gene expression analyses of mice and human ALPPS samples, as previously described.[28, 29] Human samples derived from the institutional biobank (KEK-ZH-Nr. 2015-0547) and liver biopsies from 5 patients undergoing ALPPS were analysed. Liver punch biopsies were collected from the FLR at beginning (after laparotomy) and at the end of ALPPS stage-1 operation.

Statistical analysis

R V4.0.2 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses, calculations, and graphical representations. Variables were expressed as frequencies or mean ± standard deviation (SD) or median ± interquartile range (IQR) as indicated. Significance was defined as $p<0.05$.

RESULTS

Study population

Sixty-one patients, who underwent ALPPS for various hepatic malignancies, were included for analysis (Supplementary table 1, Supplemental Digital Content 1, http://links.lww.com/SLA/E743). Median age was 59 years (IQR 47-69) and colorectal liver metastases were the most frequent entity (75%).

In total, 54 LLD were included for analysis. Median age of LLDs was 31 years (IQR 26-39) and the majority of donors were male (70%). No comorbidities were present in this highly selective cohort and postoperative morbidity was low with 13%.

Volumetric measurements

Within one week after ALPPS stage-1, median FLR volume increased from 424 mL (IQR 361-483) to 628 mL (IQR 541-753, $p<0.001$) (Table 1). Likewise, the sFLR showed a significant growth from 25.9% to 34.8% (Table 1, $p<0.001$). Despite comparable median preoperative absolute liver volume (450 mL, IQR 399-531; $p=0.278$), volume increase was significantly more pronounced after LLD as compared with ALPPS stage-1 (Table 1).

Dynamics of clinical serum phosphate levels after liver surgery

After ALPPS stage-1 and LLD, serum phosphate levels significantly dropped from preoperative median values of 1.08 mmol/L (IQR 0.92-1.23) and 1.07 mmol/L (IQR 0.91-1.21) to a postoperative median nadir of 0.68 mmol/L and 0.52 mmol/L, respectively. In both groups, the fastest phosphate drop was observed at postoperative day (POD) 2 and consequently the phosphate nadir was reached at POD 3 (Figure 1A).

In order to assess the impact of renal phosphate clearance on posthepatectomy hypophosphatemia, kidney function was assessed. Serum creatinine rate remained stable after ALPPS and LLD (Figure 1B). Serum PTH and urinary excretion of phosphate were increased within 2 days after surgery (Figure 1C/D), but returned to normal range on POD3. In contrast, serum hypophosphatemia persisted throughout the regenerative phase (Figure 1A).

Association of liver hypertrophy and phosphate drop after ALPPS stage-1 and LLD

For both, ALPPS stage 1 and LLD, degree of phosphate drop correlated with FLR volume (R=-0.43, p<0.001) and degree of hypertrophy (R=-0.40, p=<0.001; Figure 1E/F). In the ALPPS cohort, there was no significant difference in FLR hypertrophy and serum phosphate drop between the partial (n=36) and complete (n=25) ALPPS subgroup. Interestingly, phosphate drop after ALPPS stage-1 occurred in a similar way as after ALPPS stage-2 (Figure 1G), which indicates potential as an early predictor of regenerative capacity after ALPPS stage-2.

Serum phosphate drop and liver failure after ALPPS stage-2

Since only 4 patients (7%) after LLD showed mild transient liver dysfunction, association of phosphate drop and PHLF was only analyzed in patients undergoing ALPPS. Dichotomized at the median, two subgroups were formed, with a fastest phosphate drop >40%/day *versus* ≤40%/day after ALPPS stage-1. Patients with a phosphate drop >40%/day

after ALPPS stage-1 showed increased liver hypertrophy, a shorter interstage interval 8 *versus* 12 days (p=0.043), and reached completion of ALPPS stage-2 in 100% (Table 2).

As an early marker of regeneration, a phosphate drop >40% after ALPPS stage-1 was associated improved outcome in morbidity (55 *versus* 79%) and mortality (3.4 *versus* 14.3%) as well as significantly lower incidence of PHLF (Supplementary table 2, Supplemental Digital Content 1, http://links.lww.com/SLA/E743). According to ROC curve analysis, a phosphate drop >38.5%/day was the best cut-off to exclude PHLF after ALPPS stage-2 according to the 50-50 (sensitivity 78%, specificity 81%) and a drop >41.7%/day for the ISGLS criteria (sensitivity 75%, specificity 75%) (Supplementary figure 1, Supplemental Digital Content 1, http://links.lww.com/SLA/E743).

Serum phosphate and liver hypertrophy after ALPPS in mice

In mice, 80% ALPPS showed a stronger regeneration and increased liver-to-body weight ratio at 48 hours (1.39% ± 0.09%) as compared to 25% ALPPS (1.18% ± 0.10%) and PVL (0.83% ± 0.13%, p=0.023). Likewise, markers of hepatocyte proliferation (Ki67 and pH3) showed highest expression after 80% ALPPS (Supplementary figure 2, Supplemental Digital Content 1, http://links.lww.com/SLA/E743).

As in human, the strongest phosphate drop in mice was observed between 24 and 48 hours and was most pronounced in mice undergoing 80% ALPPS (-42.1% ± 9.4%) as compared to 25% ALPPS (-20.4% ± 9.3%), PVL (-6.88% ± 12.1%), or sham laparotomy (2.60% ± 4.23%). The extent of phosphate drop correlated well with the degree of hypertrophy measured as the FLR/BW volume (R=-0.69, p<0.001 Figure 2A).

Serum phosphate and liver hypertrophy after hepatectomy in mice

In order to mimic LLD, murine hepatectomy models were subsequently investigated. Phosphate drop after 68% hepatectomy (-23.0% ± 5.99%) was more pronounced as compared

to 50% hepatectomy (-8.92% ± 3.34%, p=0.012). Phosphate drop correlated well with liver hypertrophy (R=-0.88, p<0.001 Figure 2B). Interestingly, despite extended resection, a significant phosphate drop was absent in the 86% hepatectomy small-for-size model (-2.94% ±7.73%. Figure 2B), which is characterized by impaired liver regeneration. This finding supports the hypothesis, that liver posthepatectomy hypophosphatemia occurs in the regenerating liver only.

Phosphate drop and hepatic DNA replication in mice

Consequently, we aimed to investigate if FLR hypertrophy in ALPPS rodent models is owed to *de novo* DNA replication in hepatocytes. In mice undergoing 80% ALPPS, the FLR had significantly higher amounts of total double-stranded DNA content in FLR, indicating augmented DNA synthesis needed for cellular proliferation as compared to 25% ALPPS or PVL only. Further analyses revealed that a pronounced phosphate drop after surgery was an indicator for increased DNA replication (R=-0.778, p<0.001 Figure 2C), while there is also a strong Spearman correlation between FLR size and total amount of DNA (R=0.854, p<0.001 Figure 2D).

Expression of cellular phosphate transporter during liver regeneration in mice and human

Expression of cellular sodium-dependent phosphate transporter 1 (Slc20a1) was strongly upregulated in murine liver peaking at 8 hours after ALPPS/PVL (Figure 3A), as well as after hepatectomies (Figure 3B), coinciding with the time when DNA replication starts in hepatocytes. Analyzing large scale expression data of human livers at start (before transection) and end of ALPPS stage-1 surgery, we also found already a pronounced upregulation of SLC20A1 at this early time point, indicating similar mechanisms as in mice (Figure 3C).

Blockade of cellular phosphate transporter impairs hepatocyte replication *in-vitro*

Cultering of TIB-75 cells at a concentration of 10^5 in phosphate-free medium with addition of 10µM or 100µM inorganic phosphate showed a dose-dependent proliferative effect of phosphate, and absence of phosphate in the media resulted in cell death (Figure 3D). Hepatocyte proliferation was associated with cellular phosphate influx and decrease of phosphate concentration in the supernatant over time (Figure 3E). In a mechanistic experiment, addition of phosphate transport blocker, phosphonoformic acid (PFA), inhibited cellular phosphate uptake, even in the presence of 100µM phosphate, and ceased cell proliferation (Figure 3F).

DISCUSSION

For the first time, this study describes the association of serum phosphate changes and actual volume gain of the FLR after ALPPS and LLD. These clinical findings were consistent with those observed in simulated experimental murine models. Posthepatectomy hypophosphatemia is a previously described phenomenon,[5, 30-32] and the majority of studies interpret hypophosphatemia as a necessity for physiological liver regeneration.[5, 6, 33] Inevitably, restoration of liver mass is accompanied by DNA replication, leading to vast expenditure of the energy supplying molecule ATP.[6, 34, 35]

Both, ALPPS and hepatectomy for LLD are characterized by a particularly strong regenerative impulse.[8, 9, 11] This study found that rapid liver hypertrophy after ALPPS and LLD translates into profound serum phosphate expenditure. Serum phosphate levels followed a strict orchestration, with the most pronounced drop between 48-72 hours, which coincides with the mitotic peak at 48-72 hours after liver resection, as observed by other authors.[6, 36] Another group described comparable phosphorus shifts during liver regeneration utilizing

phosphorus 31 nuclear magnet resonance spectroscopy.[6, 36] In this line, animal and *in vitro* experiments of this study, not only supported the kinetics of serum phosphate in the clinical scenario of ALPPS and LLD, but also indicated cellular phosphorus ingestion and increased expression of cellular phosphate transporter translating in *de novo* DNA synthesis and FLR parenchyma growth under regenerative conditions. Furthermore, our observation of absence of hypophosphatemia in a murine small-for-size model of 86% hepatectomy underlines the important indicator function of postoperative hypophosphatemia in liver regeneration.

In parallel, liver regeneration triggered also early postoperative transitory increase of serum PTH with associated hypophosphaturia. This phenomenon known as liver-kidney axis in hepatectomy-related hypophosphatemia has been reported before.[37, 38] While PTH and urinary phosphate excretion normalized at day 3 after surgery, serum phosphorus levels remained low throughout the whole initial regenerative phase. Therefore, liver regeneration appears to be the dominant phosphate consumer for hepatectomy-related hypophosphatemia although the exact contribution of postoperative hyperphosphaturia remains unclear. However, others suggest that post-hepatectomy hypophosphatemia primarily relies on the action of phosphaturic factors.[37-40]

In the literature, there are contrary reports on the effects of hypophosphatemia following major hepatectomy.[41] While hypophosphatemia was associated with inferior outcome after hepatectomy in some studies[31, 33, 42, 43 44, 45], other reports including ours demonstrated superior outcome of postoperative hypophosphatemia along with poor outcome in absence of postoperative hypophosphatemia.[3, 32, 46] A recent study including 749 patients, revealed serum phosphorus >2.4mg at day 2 after major hepatectomy as an independent predictor of liver failure, postoperative complications, and mortality.[32] Another group described serum phosphate levels ≤0.65 mmol/L as a beneficial marker for recovery from PHLF.[3] Of note, this

cut-off value corresponds to the median phosphate nadir of 0.66 mmol/L observed in our study, which supports the universality of our findings. These clinical observations go in line with the findings of the present study that hypophosphatemia indicates regeneration while absence or delayed onset of hypophosphatemia should alert clinicians to impaired liver regeneration and elevated risk of developing PHLF. Of note, PHLF criteria are usually applied on POD 5 [16, 17] and meaningful image-based volume growth cannot be assessed before POD 5.[4, 8, 47] Therefore, early postoperative hypophosphatemia on POD 2-3 has the great advantage to predict liver hypertrophy before sufficient volume increase can be imaged or PHLF criteria can be applied.

Whereas, adverse events and liver failure in particular are extremely rare after LLD, the ALPPS procedure can be accompanied by relevant morbidity, in particular after completion ALPPS stage-2. Since phosphate drop after ALPPS stage-1 correlated well with phosphate changes after ALPPS stage-2, it was tested as a surrogate marker for the regenerative capacity. Indeed, phosphate drop after ALPPS stage-1 showed potential as an early discriminator of PHLF after ALPPS stage-2. In future, considering phosphate changes after ALPPS stage-1, may help to discriminate individuals who are prone to develop complications after ALPPS stage-2 at an early stage, prior to exposing patients to a potentially harmful procedure. Based on the findings of this study, a low phosphate drop after ALPPS stage 1 may encourage surgeons to perform more detailed examination of FLR function, or even to postpone early on the second stage and allow a more pronounced liver regeneration prior to resection.

The major strength of our study relates to the availability of volumetric imaging at a defined time point one week after ALPPS stage-1 and LLD in all patients enabling highly standardized assessment of comparable liver volume growth. Further, translational

experiments support the observation in humans and mechanistic approaches emphasized the role of phosphate drop in liver regeneration. The study was also associated with limitations, which are mainly related to retrospective design, and the lack of standardized phosphate substitution. Nevertheless, the impact of this substitution bias on phosphate drop is neglectable since phosphate replacement was performed in a reactive fashion only and did not influence the initial drop. Additionally, given the sample size, the study may be underpowered to highlight certain differences at a level of statistical significance.

In conclusion, this study provides evidence for the first time that early serum phosphate drop after liver surgery is a direct surrogate marker for liver hypertrophy. Perioperative implementation of serum phosphate analysis has the potential to detect patients with insufficient regenerative capacity at an early stage before sufficient volume increase can be imaged or posthepatectomy liver failure criteria can be applied.

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Figure 1. *Serum phosphate drop, kidney function, and liver hypertrophy after ALPPS and Living Liver Donor Hepatectomy*. Panel A and B illustrate postoperative serum phosphate and creatinine levels after ALPPS stage-1 (*circle, black*) and living donor hepatectomy (*circle, gray*). Panel C and D show postoperative serum PTH levels and urine phosphate excretion in living donors (n=15). Upper and lower reference ranges are illustrated in panels A-D. Scatter plots of (E) FLR volume increase *versus* serum phosphate drop at POD 2 and (F) liver hypertrophy in percent *versus* phosphate drop at POD 2 for ALPPS (*circle, black*) and living donors (*circle, gray*). Panel G shows scatter plot of serum phosphate drop at POD 2 for ALPPS stage-1 *versus* ALLPPS stage-2. Spearman correlation coefficients (R) are presented along with *p*-values. ALPPS indicates Associating Liver Partition with Portal vein ligation for Staged hepatectomy; FLR, future liver remnant; POD, postoperative day; PTH, parathormone.

Figure 1

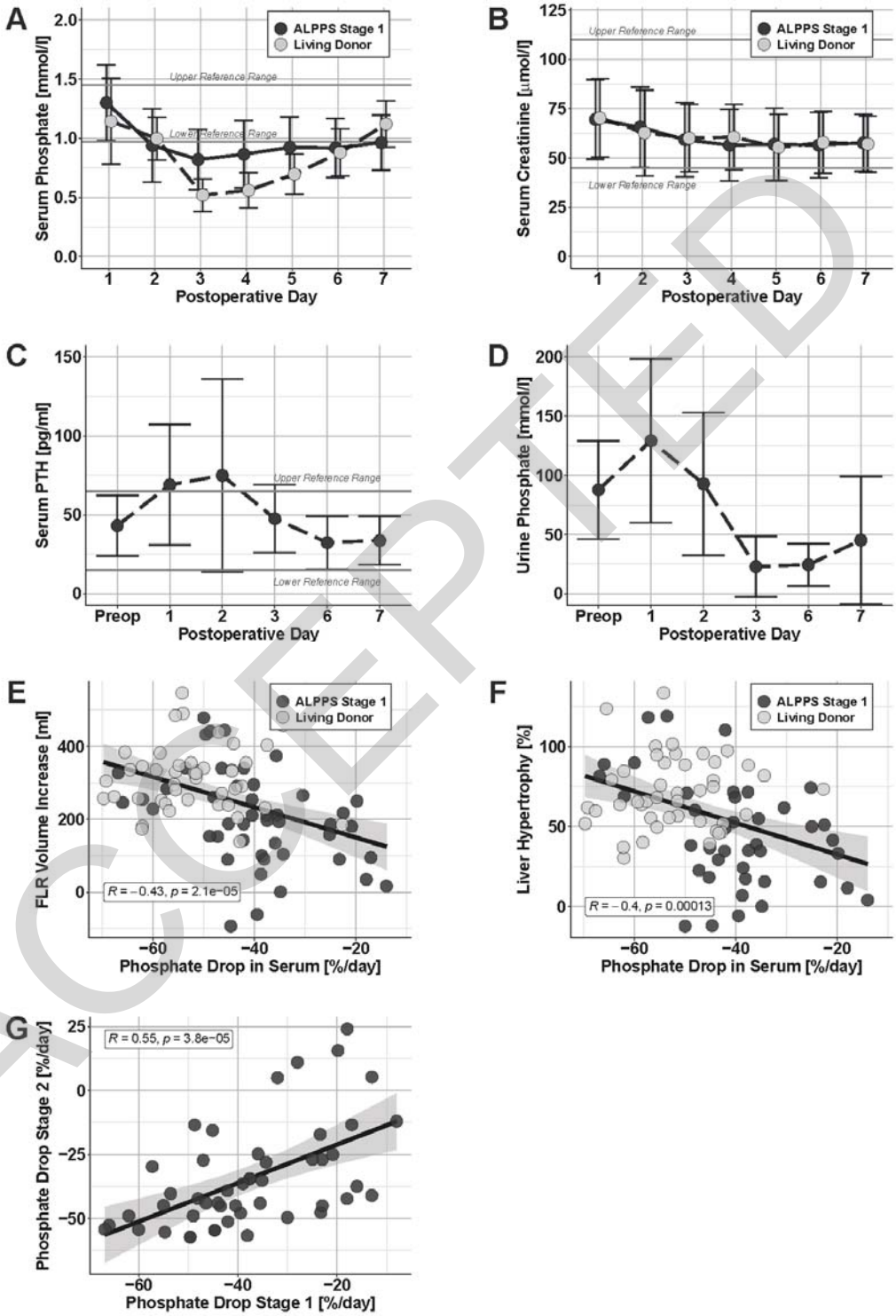

Figure 2. *Experimental model of hypophosphatemia and liver regeneration*. Serum phosphate drop and FLR growth were studied in ALPPS (A, C, D) and hepatectomy (B) murine model at POD 2. The ALPPS model included various degrees of transection (0%=PVL, 25%, 80%) and the hepatectomy model various degrees of hepatectomy (50%, 68%, 86%). The 86%-hepatectomy model represents a non-regenerative model. In both models, sham operation served as control. Figure panels A/B show the correlation between serum phosphate drop gain of liver mass after ALPPS (A) and hepatectomy (B). Additional analysis of DNA amount in the regenerating FLR after ALPPS revealed highest DNA levels after 80% transection (C). The DNA amount correlated well with the degree serum phosphate drop (C) and liver hypertrophy (D). Spearman correlation coefficients (R) are presented along with *p*-values. ALPPS indicates Associating Liver Partition with Portal vein ligation for Staged hepatectomy; FLR, future liver remnant; POD, postoperative day; PVL, portal vein ligation.

Figure 2

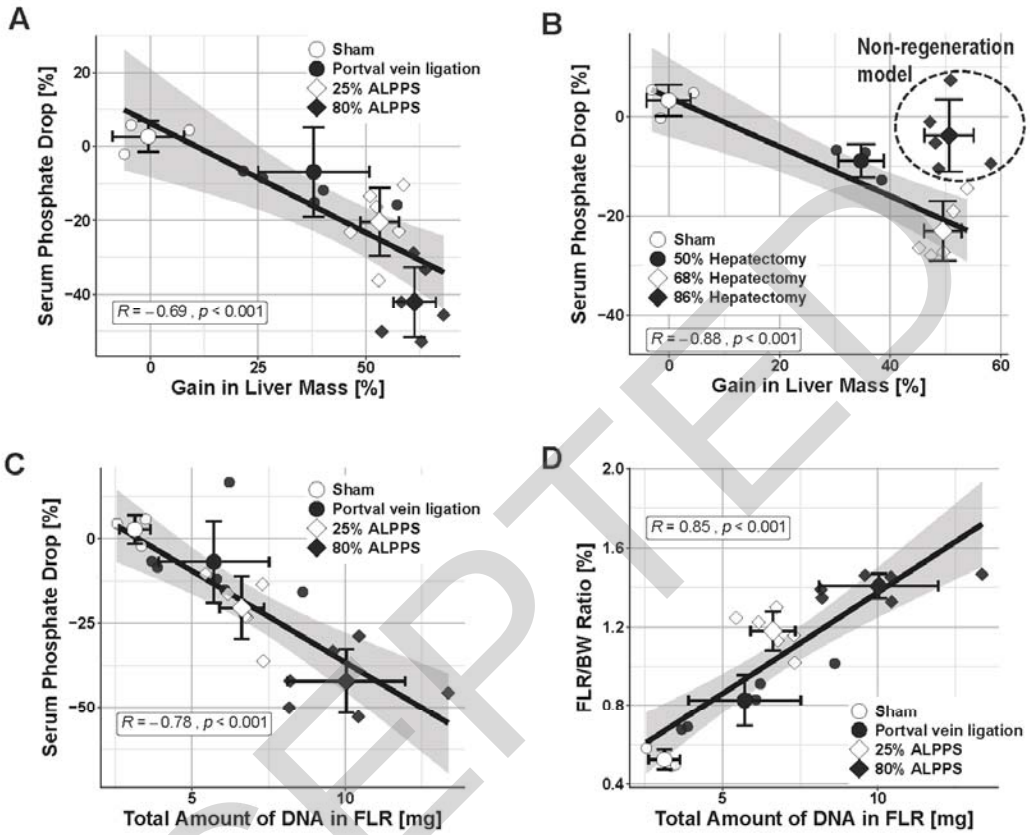

Figure 3. *Cellular phosphate influx through phosphate transporter under in-vitro growth conditions.* Expression of cellular sodium-dependent phosphate transporter (*Slc201a1*) was strongly upregulated during regeneration 8 hours after ALPSS/PVL (A) and hepatectomy (B) in mice. Same pattern was observed in tissue samples of human liver parenchyma of patients undergoing ALPPS stage-1 (n=5), with a significant increased expression of *SLC201A1* at the end of ALPPS stage-1 surgery (C). Cultering TIB-75 hepatocytes with 10µM or 100µM of inorganic phosphate resulted in a dose dependent acceleration of cell replication whereas absence of phosphate in culture media resulted in cell death (D). Culturing hepatocytes in media with 100 µM phosphate resulted in an increased cell number and simultaneous decrease of phosphate concentration in culture media (E). Addition of 1 mM phosphorformic acid (PFA), an inhibitor of phosphate transport, stopped cellular phosphate uptake and cell proliferation (F).

Figure 3

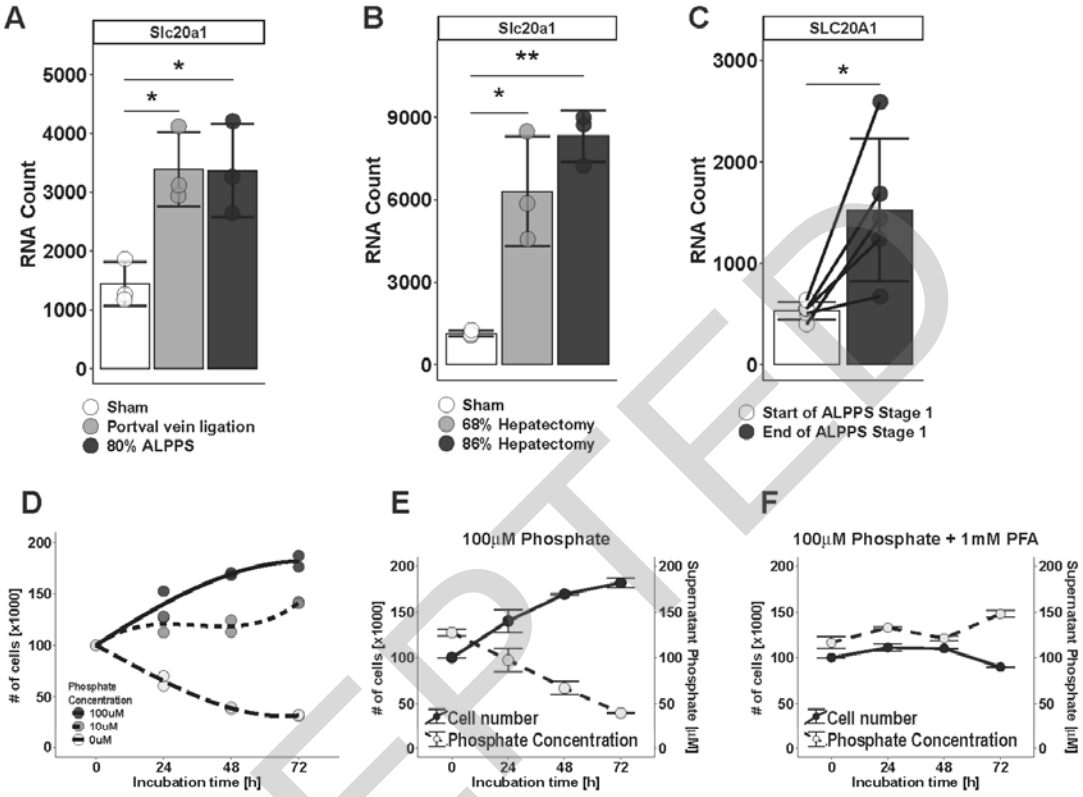

Table 1. Volumetric measurements in ALPPS patients and living donors

Volume characteristics	ALPPS stage-1 (n=61)	LLD (n=54)	*P*
Volume changes in mL			
FLR, preoperative [mL]	424 (360-483)	450 (399-531)	0.278
FLR, postoperative day 7 [mL]	627 (541-753)	752 (654-843)	0.028
Volume changes in %			
sFLR, preoperative [%]	25.9 (20.4-32.9)	32.1 (31.4-36.3)	0.132
sFLR, postoperative day 7 [%]	34.8 (29.6-41.2)	41.8 (39.3-45.3)	0.037
sFLR volume kinectics			
FLR volume increase [mL]	212 (135-282)	284 (239-332)	0.021
Degree of hypertrophy [%]	57.5 (29.7-80.0)	57.6 (52.7-73.3)	0.523
Kinetic growth rate [%/d]	6.9 (3.0-10.5)	8.2 (7.5-10.5)	0.021

Data are presented as median and interquartile range
Abbreviations: ALPPS, Associating Liver Partition with Portal vein ligation for Staged hepatectomy; FLR, Future liver remnant; LLD, living liver donation; sFLR, standardized future liver remnant

Table 2. Phosphate drop and outcome after ALPPS stage-1 and stage-2

Variable	High phosphate drop >40%/day (n= 29)	Low phosphate drop ≤ 40%/day (n=32)	Total (n=61)	*P*
ALPPS stage-1				
Phosphate pre stage-1 [mmol/L]	1.06 (0.9-1.2)	1.14 (1.00-1.18)	1.08 (0.94-1.19)	0.954
Phosphate drop after stage-1 [%/d]	-48.7 (-55.9-44.7)	-34.3 (-37.6--23.1)	- 40.3 (-68.9--17.3)	<0.001
ALPPS stage-2				
Phosphate pre stage-2 [mmol/L]	1.02 (0.90-1.10)	1.09 (1.00-1.13)	1.04 (0.91-1.21)	0.876
Phosphate drop after stage-2 [%/d]	-48.1 (-54.1--37.7)	-34.5 (-46.3--8.9)	- 42.3 (-54.6--34.9)	0.025
Interstage Interval [days]	8 (6-10)	12 (9-16)	9 (7-15)	0.043
ALPPS completion rate, % (n/N)	100 (29/29)	87.5 (28/32)	93.4 (57/61)	0.675
Interstage complications,	24.1 (7/29)	21.9 (7/32)	22.9 (14/61)	0.496
% (n/N)	17.1 (5/29)	12.5 (4/32)	14.8 (9/61)	0.691
Minor complications ≤3a, % (n/N)	7.0 (2/29)	9.4 (3/32)	8.2 (5/61)	0.987
Major complications ≥3b, % (n/N)				
Volumetric changes				
Kinetic growth rate [%/d]	7.2 (5.1-11.6)	5.5 (2.6-8.4)	6.9 (3.0-10.5)	0.096
FLR volume increase [mL]	264 (196-347)	183 (112-211)	212 (135-282)	0.019
Degree of hypertrophy [%]	70.9 (49.1-89.5)	39.0 (15.7-55.1)	57.5 (29.7-80.0)	0.018

Data are presented as number (percent) or median with interquartile range
Abbreviations: ALPPS, Associating Liver Partition with Portal vein ligation for Staged hepatectomy; PHLF, Posthepatectomy liver failure, FLR: Future liver remnant

#03

ANNSURG-D-23-00414	**Henrik Petrowsky**	**Early postoperative serum phosphate drop predicts sufficient hypertrophy after liver surgery**	**ESA Paper**

First Discussant: Christophe Laurent (Bordeaux, France)

I would like to thank the ESA for the privilege of being the first discussant of this paper, and the authors for this interesting study. Post-hepatectomy liver failure is one of the leading causes of post-operative mortality. Understanding the causes of non-regeneration of the liver, despite the implementation of pre-operative preparations, such as ALPPS, portal vein embolization and porto-sus hepatic bi-embolization, remains a challenge. The objective of this study was to analyze the impact of serum phosphate level evolution on liver regeneration after ALPPS and hepatectomy in the living donor. A significant decrease in serum phosphate level after ALPPS stage 1 would be a good indicator of liver regeneration, with a possible decrease in morbimortality during stage 2 of the procedure. However, in a recent review of the literature on this topic, the impact of hypophosphatemia on post-hepatectomy liver failure remains unclear because few studies have associated the analysis of liver regeneration with the degree of hypophosphatemia.

My first question is: In your analysis, why did you not include known preoperative factors, aside from the evolution of phosphatemia, that may have a possible effect on liver regeneration, such as those related to the patient (male sex, advanced age, obesity) and those related to the liver parenchyma (cirrhosis, fibrosis, steatosis, preoperative chemotherapy, cholestasis), in order to determine the actual prognostic impact on liver regeneration? Can you clarify this point, as it might impact the conclusions of your study?

Second, in your study, you included two very different groups of patients: ALPPS (a validated technique for insufficient future liver volume) and living donors, where the objective is to limit the risk of liver failure as much as possible, and therefore, have sufficient residual liver volume. It is certainly a major hepatectomy, but in a totally different pathological context. Moreover, the association between a phosphate drop and post-hepatectomy liver failure was only studied in patients with ALPPS because there were not enough events in the living donor group, with only 7% of moderate liver dysfunction. In order to have homogeneity in the studied populations, why did you not analyze the evolution of phosphatemia after ALPPS stage 1 and porto-sus-hepatic bi-embolization, which are two procedures generating a strong hepatic hypertrophy that is recommended for patients with a future liver volume remaining below 25%?

Third, during the preparation by porto-sus hepatic bi-embolization, it is well-specified that a vitamin and phosphate supplementation protocol should be followed to promote the DNA replication necessary for liver regeneration. The phosphate supplementation protocol proposed in this study is far from homogeneous since the living donors did not have

substitution and only some ALPPS had it. Can you clarify this point, which could represent a bias for the interpretation of the results?
Finally, you suggest that "a low phosphate drop after ALPPS stage 1 may encourage surgeons to perform a more detailed examination of future liver remnant function". In order to analyze this insufficient hepatic regeneration, can you specify where the liver biopsy was done, in addition to the 99mTc-mebrofenine hepatobiliary scintigraphy?

Response From Patryk Kambakamba (Zurich, Switzerland)
Thank you for your valuable comments. First, with regards to other pre-operative factors, I completely agree that a holistic assessment and understanding of patient characteristics is of utmost importance for any type of surgery. In fact, we have displayed these variables in the supplementary table.
However, we think that the beauty of the phosphate drop is that it predicts liver regeneration, regardless of these potential risk factors or the quality of the liver. In other words, if you have a patient with a cirrhotic or fibrotic liver, the phosphate drop will be lower; whereas, if you have young, healthy liver donors, the phosphate drop will be higher. To conclude, I think these factors certainly need to be considered, but I doubt they would have a big impact on the results of our study.
Second, of course, it would be interesting to study phosphate changes after double vein embolization. However, we decided to investigate the phosphate drop in the setting of two procedures, which are described to induce the most pronounced liver regeneration. Therefore, the ALPPS procedure and living liver donation, which showed excellent liver growth, in our experience, were chosen. Additionally, validated animal models for both procedures were previously established by our study group. This allowed translational experiments with novel mechanistical insights.
Third, we agree that it would be valuable to have a standardized phosphate protocol. However, up until now, the effect of phosphate supplementation on liver regeneration has been controversial, and no validated supplementation protocol exists. In line with this, the living donor group showed an excellent regeneration, despite the lack of phosphate supplementation. In fact, only a minority of ALPPS patients had phosphate supplementation, which was administered in a reactionary fashion after the phosphate drop had already occurred. Likewise, none of the operated mice received phosphate, and still, regeneration remained unaffected. Therefore, in the future, I think it would be very interesting to not only observe the phosphate drop but also the dynamic of recovery from the drop by supplementation.
Finally, regarding the role of biopsy, I completely agree that there is room for biopsy and a more sophisticated investigation. However, the question is more about determining which patients you should select for this. In this context, the phosphate drop is obviously a very cheap investigation, which may help to determine who would benefit from a more profound investigation of liver quality and function. Therefore, the phosphate drop could potentially increase cost-effectiveness and efficiency when using resources, such as a biopsy or HIDA scan.

Discussant: Antonio D. Pinna (Weston, United States)

This study is very interesting. First, did you correlate the drop in the phosphate with the volume of the liver? If there is a correlation, which do you prefer to follow to predict potential liver failure after Stage 1: the volumetry or the drop in the phosphate?
Also, you don't need to perform a biopsy prospectively. You can just look at the piece of liver you removed and see whether there is a correlation between the phosphate drop and the histology of the liver. If you have a population of living donors, you can easily compare this.

Response From Patryk Kambakamba (Zurich, Switzerland)
These are all very good points. I'll begin with your last comment. This would certainly be feasible in a population of living donors, or even in the ALPPS group, where we obviously have biopsy material, especially if we do a cleaning of the FLR in the first stage. However, essential information on histological liver changes during liver regeneration would still be lacking.
Regarding your first question, indeed, the phosphate drop showed a linear correlation with liver growth in humans and mice. Still, at that stage, it is too early to rely solely on the phosphate drop, since these findings need to first be validated in a bigger cohort. However, when we plan surgeries, we already take the phosphate drop into consideration, in addition to other established factors, such as histology and volumetry. We all know the dilemma: volume alone does not sufficiently predict function and the potential for regeneration. In that context, the phosphate drop may be an additional puzzle piece for a more precise understanding of regenerative capacity.

Discussant: Jacques Pirenne (Leuven, Belgium)
Isn't phosphate necessary for the regeneration of other types of cells? Do you have any data on the drop of phosphate, for example, after intestinal ischemia or regeneration in other settings?

Response From Patryk Kambakamba (Zurich, Switzerland)
Exactly, phosphate changes have been pre-described studies for other types of surgery. However, the process of regeneration by tissue gain is a unique characteristic of the liver. Therefore, hypophosphatemia plays a particular role after liver surgery.

Discussant: Christiane Bruns (Cologne, Germany)
Thank you for a great presentation and excellent work. You described a decrease in serum phosphate levels after ALPPS Stage 1 as an early event and good indicator of liver regeneration, with a possible decrease in morbidity and mortality during ALPPS stage 2. Furthermore, you postulate, that in the case of a low decrease in serum phosphate levels and associated reduced liver regeneration, you would suggest waiting for a longer period to give the liver a chance to regenerate more. If your stimulus for good liver regeneration is an early decrease in serum phosphate levels after ALPPS Stage 1, then why should the liver then regenerate better when waiting longer? I don't understand your conclusion.

Response From Patryk Kambakamba (Zurich, Switzerland)

Thank you for this question. This is true; however, if you observe the evolution of ALPPS, in the beginning, we had a frighteningly high rate of mortality. Then, we assessed patients better and had an enhanced understanding of patient selection. Another factor that made ALPPS safer was, indeed, waiting longer. Whereas in the earlier days of ALPPS, we aimed for an interval period of around 1 week; in the later days, the median was around 10-12 days or even longer. In my opinion, the biggest benefit of ALPPS is resectability, which is around 90%. We should not rush to resect a "premature" liver as soon as possible. I really think that a part of making the procedure safer is giving the liver more time to grow.

Discussant: Elisabeth Nieveen van Dijkum (Amsterdam, The Netherlands)
As I'm more endocrine-interested, I was triggered by your phosphate. I'm also a little bit involved with the liver team at our clinic, and we have done some research on the aldosterone levels post-surgery. If you compare the PTH to the phosphate, perhaps, you are losing some information. The kidneys' production of aldosterone is highly involved in the first part of liver operations. Already during surgery, we see enormous levels of aldosterone; therefore, I think the system is not only dependent on your PTH but also on the renal system. Have you measured any urinary output of aldosterone, especially in the animal studies?

Response From Patryk Kambakamba (Zurich, Switzerland)
Thank you for this valuable comment. My answer is short. We did not measure this, but this could be useful for future studies.

Discussant: Ricardo Robles-Campos (Murcia, Spain)
The surgeons we are looking at are continuing to avoid acute liver failure after the second stage, mainly in the ALPPS surgical technique. We perform our criteria by analyzing the function of the liver before the second operation. Did you correlate the scintigraphy with the serum phosphate levels after the first stage of ALPPS?

Response From Patryk Kambakamba (Zurich, Switzerland)
Indeed, we have scintigraphy results for a subset of these patients, but we have not yet correlated HIDA scan data with phosphate changes. However, this is a good point, and it may become the topic of future investigations.