Title: Pharmacodynamic Analysis Of A Fluid Challenge.

Short Running title: Pharmacodynamics of a fluid challenge

Hollmann D. Aya (MD)[1], Irina Chis Ster (PhD)[2], Nick Fletcher (MD)[1], R. Michael Grounds (MD-Res)[1], Andrew Rhodes (MD-Res)[1], Maurizio Cecconi (MD-Res)[1]

1: Adult Critical Care Directorate, St George's University Hospitals and St George's University of London

2: Senior Lecturer in Biostatistics, Institute of Infection and Immunity, St George's University of London

Institution: St George's University Hospitals, NHS Foundation Trust.

Address: General Intensive Care Unit, St James Wing, First floor.
             St George's University Hospitals, NHS Foundation Trust.
             Blackshaw road, SW17 0QT, London, UK

Corresponding author: Hollmann D. Aya, email: hollmann.aya@nhs.net

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Key Words
- Fluid challenge
- Haemodynamics
- Pharmacodynamics
- Mean systemic filling pressure
- Stress-relaxation
- IV Fluids

Abstract

Objective: This study aims to describe the pharmacodynamics (PD) of a fluid challenge over a ten-minute period in post-operative patients.

Design: Prospective observational study

Setting: General and cardiothoracic intensive care unit, tertiary hospital.

Patients: 26 post-operative patients.

Intervention: 250 ml fluid challenge performed over 5 minutes. Data was recorded over 10 minutes after the end of fluid infusion

Measurements and main results: Cardiac output (CO) was measured with a calibrated LiDCO*plus*™ (LiDCO, UK) and Navigator™(Applied Physiology, Australia) to obtain the Pmsf analogue (Pmsa). PD outcomes were modelled using a Bayesian inferential approach and Markov chain Monte Carlo estimation methods. Parameter estimates were summarized as the means of their posterior distributions and their uncertainty was assessed by the 95% Credible Intervals (CrI). Bayesian probabilities for groups' effect were also derived. The predicted maximal effect on CO was observed at 1.2 minutes (95% CrI -0.6 to 2.8) in responders. The probability that the estimated area under the curve of central venous pressure (CVP) was smaller in non-responders was 0.12. (estimated difference -4.91 mmHg·min (95% CrI –13.45 to 3.3). After 10 minutes, there is no evidence of a difference between groups for any haemodynamic variable.

Conclusion: The maximal change in CO should be assessed one minute after the end of the fluid infusion. The global effect of the fluid challenge on CVP is greater in non-

responders but not its change ten minutes after the fluid infusion. The effect of a fluid

challenge on hemodynamics is dissipated in ten minutes similarly in both groups.

Introduction

The administration of intravenous (IV) fluids is essential for the management of critically ill patients. In order to reduce any undesirable effects from the inappropriate use of fluids (1-4), the fluid challenge technique has been recommended (5, 6) and it is one of the commonest interventions in intensive care. A fluid challenge is a test in which a small bolus of IV fluid is given over a short period of time to assess haemodynamic response (7) and it is considered as the "gold standard" test for assessment of fluid responsiveness (8, 9).

Weil and Henning (5) proposed that an increase of CVP greater than 2 cm H_2O sustained over 10 minutes indicates that no additional fluid should be given. If it declines, the fluid challenge should be resumed. As far as we know, this concept has not been tested. Moreover, despite the fact that a fluid challenge is a very common practice, there is little agreement regarding how to perform it: reviews of the literature show marked heterogeneity of triggers, volume infused, time of assessment or variable targets (10, 11). Recently, a multicentre international observational study assessed the way a fluid challenge is performed and the results highlight the great variability in terms of volume used, rate of infusion, timing of the measurements and interpretation of results (12). The authors highlight the need of more research in order to standardise this technique.

Guyton (13) observed that an acute expansion in blood volume increases the mean systemic filling pressure (Pmsf) and generates a progressive stretching of the vascular system so that, after some minutes, the Pmsf falls back to the baseline level, in a similar fashion to CO, despite the expansion of circulating volume (13). Pmsf is the pressure generated by the volume within the cardiovascular system under static conditions (no blood motion) (14). Pmsf depends on the mean compliance of the cardiovascular system

and the intravascular volume and is a key determinant of both venous return (VR) and CO. Guyton's observations suggest that a very rapid stress relaxation occurs in the circulatory system following the expansion of intra-vascular volume and, as a consequence, the effect of the fluid challenge may be rapidly dissipated.

The objective of the present study is to describe the pharmacodynamics of a fluid challenge across several haemodynamic variables and to explore the differences between responders and non-responders in a group of post-operative patients.

Material and Methods

The National Research Ethics Service Committee considered this study a service evaluation and it was approved by the institutional Joint Research and Enterprise Office; therefore no written informed consent was required.

This is a prospective observational study performed in the general and cardiothoracic intensive care units (ICU), of a tertiary university hospital between November 2011 and September 2014. Post-operative patients admitted to the ICU and receiving a fluid challenge in accordance with our goal-directed therapy protocol (ESM Appendix 1) (15, 16) were eligible for this study. Patients without a central venous catheter, known or post-operative aortic valve regurgitation, presence of an intra-aortic balloon pump, known pregnancy, body weight less than 50 kg, known or suspected sepsis and patients in haemorrhagic shock requiring blood products were excluded. In addition, patients with perioperative echocardiographic evidence of severe right or left ventricular dysfunction and patients who required aggressive fluid resuscitation or changes in sedo-analgesia, vasoactive therapy or respiratory support during the period of study were also excluded. The study period was not initiated until the haemodynamics were in a steady state - defined by changes in mean arterial pressure, heart rate and CO no greater than 10 % during 10 minutes before data recording. Patients received one or more fluid challenges according to the clinical prescription.

Cardiovascular monitoring

Patients had continuous arterial blood pressure monitoring from a radial artery catheter (115.090 Vygon, Ecouen, France). CVP was measured with a venous central catheter (CV-15854, Arrow International, Reading, USA) inserted into the internal jugular or the subclavian vein. Both catheters were connected to a pressure transducer

(T001650A, Edwards Lifesciences LLC, Irvine, USA) and to a multi-parameter monitor (Infinity™ Delta, Drager Medical Systems, USA). Zero levels for pressure measurements were referenced to the intersection of the anterior axillary line and the fifth intercostal space.

CO was measured with the LiDCO™*plus* system (17) (LiDCO Ltd, Cambridge, UK) calibrated with an injection of lithium chloride (0.3 mmol) given according to the manufacturer's recommendation (18, 19). Beat-to-beat CO and stroke volume (SV) was obtained with LiDCO™*plus* pulse power analysis(20).

Determination of Pmsf-analogue: Pmsa

The Navigator ™ software system (Applied Physiology, Sydney, Australia) was connected to the multi-parameter monitor and to the LiDCO™*plus.* Pmsa calculation is based on the values of CO, CVP, mean arterial pressure (MAP), and patient's anthropometric measures (height, weight and age) (21, 22).

Fluid challenge

The fluid challenge consisted of 250 ml of crystalloid (Compound sodium lactate, Baxter Healthcare Ltd, U.K) infused using a syringe of 50 ml and performing 5 boluses over 5 minutes. According to the clinical protocol, an increase in CO immediately after the fluid challenge greater than 10% was considered a positive response (R). The values recorded at baseline and immediately after the fluid challenge were used for this classification.

Pharmacodynamic analysis

Haemodynamic values were recorded electronically during the whole study period in a log file. The data uploaded from the LiDCO*plus*™ monitor was set to record at a beat to beat basis, and the Navigator™ monitor recorded a data sample of all variables every 10

seconds. The data for analysis were obtained at base line, at the end of the infusion, and 1, 2, 4, 6, 8, and 10 minutes after the end of fluid infusion.

Several variables of interest were defined as outcomes to describe the effect of a fluid challenge on haemodynamic variables: the global effect over ten minutes can be quantified as the net area under the curve (AUC) calculated using the trapezoidal rule from the baseline value (23). In addition the maximal difference from baseline observed (d_{max}), maximal value observed (E_{max}), time when the maximal value was observed (t_{max}) and change from baseline at 10 minutes time (d_{10}) are also reported.

Statistical analysis

The data was explored graphically and summarized according to its nature, i.e. means, medians, interquartile range (IQR) and standard deviation (SD) for continuous variables and percentages for categorical/binary variables. Classical frequentist approaches such as Kruskal-Wallis equality-of-population rank test and Fisher's exact test were implemented for independent data (baseline measurements) and results assessed through classical p-value with values below 0.05 deemed as statistically significant. Each patient is subjected to one or more fluid challenges and that can result in multiple measurements per individual. Hence, the data exhibit a hierarchical structure with two levels of variability, which need to be accounted for: *between subjects* as a well as *within subject* variability.

A random slopes modeling framework allows each individual's slope (which reflects the association between an individual outcome (say AUC Pmsf) with the corresponding individual measurements) to vary. The inference consists in estimating an *average line* (defined by an average intercept and an average slope), reflecting the association of the outcome with baseline measurements by clinical group (i.e. responders and non-responders) as well as the average value of the outcome for an average baseline

measurement. In other words, we understand the average group behavior accounting for individuals' variability.

A Bayesian framework for statistical inference and Monte Carlo Markov chains (MCMC) methods were implemented. Unlike the frequentist approach, the parameter values are random variables rather than numbers and therefore summarized by their means and 95% credible intervals (CrI) of their *posterior* distributions and reported accordingly. Unlike the classical 95% confidence intervals, the 95% CrI can be interpreted as the 95% chance that the mean belongs within its limits. No prior knowledge was assumed for any of the parameters, which included the estimated variances. In order to quantify the extent to which the two groups differ with respect to their outcome, the probability that the mean outcome in responders is greater (or smaller) than in non-responders was calculated. We shall refer to this as to the Bayesian probability of the group effect to avoid confusion with the classical p-value. The sense of interpretation depends on the clinical connotation. Probabilities smaller than 0.05 and greater than 0.95 were considered as strong evidence. Probabilities smaller than 0.21 and greater than 0.79 were deemed as fairly good evidence.

Two sets of statistical models of increasing complexity were fitted to data. One set labelled as a *simple models* involves two main parameters of interest: one quantifies the difference between responders and non-responders ($\Delta(R - NR)$) and the second one the average change in outcome for one unit increase in the baseline of each haemodynamic variable *irrespective* the group (R or NR). The other set called interaction models explores the possibility that the average changes in outcomes for one unit increase in the baseline may differ across the two groups of patients.

Mean pharmacodynamics outcomes are the predicted after parameter estimation for each group, for an average baseline value following inference from the interaction

models set. The deviance information criterion (DIC) has been employed to assess choose between models of different fit – the smaller the value, the better the fit. However, model choice has been also subjected to clinical considerations rather than strictly following formal statistical rules.

Statistical software used included OpenBUGS (24, 25), STATA (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP) and R (R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Austria. URL http://www.R-project.org/)

Results

50 fluid challenges were observed in 26 patients. Demographic and baseline data is presented in Table 1. The median (IQR) number of fluid challenges per individual was 2 (1 – 2) with 1 (1 – 2) in non-responders and 2 (1 – 3) in responders. 13 (50%) patients were responders. The median time between fluid challenges was 27 minutes (18 – 43). In 2 patients a different response in CO was observed after the initial fluid challenge. From the total number of events, 26 (52%) were responders. The median fluid infusion time was 3.4 (2.6 – 4.1) minutes.

Baseline and demographic data was not significantly different between groups (Table 1) except for the ICNARC score, which did not reveal a significant effect (the CrIs approximately evenly spread around 0 when model was taking in account ICNARC values). The results are presented according to the interaction model although some of the results were not statistically superior but physiologically consistent. Results are summarised in Tables 2 and 3. For all the variables, an increase in baseline corresponds with an increase in the estimated maximal value (E_{max}).

Mean arterial pressure

The estimated global effect of the fluid challenge (AUC) is similar in both groups (table 2), however, in responders the maximal effect was achieved faster (1.58 min (95%CrI -0.15 to 3.31) vs. 4.5 min (95% CrI 2.7 to 6.3) Probability of Δ(R-NR)>0 = 0.01). The higher MAP at baseline, the smaller AUC and d10 in both groups. However, the higher MAP at baseline, the smaller is dMax in responders and the shorter the time to reach it. (Table 3, Figure 1 and 2).

Cardiac output

In responders the estimated AUC was greater (estimated AUC Δ(R-NR) 1.9 L (95% CrI - 0.7 to 4.5), probability Δ(R-NR)> 0 = 0.93), the maximal effect on CO was greater (estimated d_{max} Δ(R-NR) 0.29 L/min (95% CrI -0.20 to 0.75), probability Δ(R-NR)> 0 = 0.89), it occurs faster (estimated t_{max} Δ(R-NR) -2.61 min (95% CrI -4.86 to -0.39), probability Δ(R-NR) > 0 = 0.01) and the estimated maximal value was greater than in non-responders (estimated E_{max} Δ(R-NR) 0.28 L/min (95% CrI -0.20 to 0.74), probability Δ(R-NR) >0 = 0.88). Importantly, the maximal effect was observed one minute after the end of the fluid infusion (1.16 minutes (95% CrI -0.56 to 2.84). In both groups, CO returns to base line similarly at 10 minutes time point. Patients with higher CO at baseline reach the maximal effect quicker regardless of the CO response group (Table 3).

Mean systemic filling pressure analogue

The estimated AUC is similar in both groups (estimated Δ(R-NR) -1.52 mmHg (95% CrI - 8.8 to 5.5), probability Δ(R-NR) >0 = 0.34), although the responders achieved the maximal effect quicker than non-responders. In non-responders there is no relationship between Pmsa baseline and AUC or dMax, but in responders there is a negative relationship so the higher Pmsa at baseline the smaller estimated AUC and smaller estimated dMax on Pmsa (Table 3, Figure 3 and 4).

Central venous pressure

The estimated AUC is greater in non-responders (estimated Δ(R-NR) -4.91 mmHg (95% CrI -13.45 to 3.3), probability Δ(R-NR) >0 = 0.12), although none of the other outcomes achieved a good level of evidence in terms of difference between groups.

Those non-responders with higher CVP at baseline had a shorter time to observe the maximal value on CVP (Table 3, figure 5). In responders the increase in CVP at baseline increased the effect observed at 10 minutes time (Figure 6)

Heart rate (HR)

The global effect was similar in both groups (estimated Δ(R-NR) -3.71 bpm (95% CrI -23.39 to 15.69) probability Δ(R-NR)>0 = 0.35), as well as the other outcomes. Patients with higher HR at baseline showed a decreased AUC in both groups (Table 3). DMax and d10 becomes greater (more negative) only in responders (Figure 7 and 8) as long as the baseline HR increases.

Discussion

The main findings of this study are, first, the maximal change in CO is observed one minute after the end of fluid infusion; second, the global effect of the fluid challenge on CVP is higher in non-responders but not the change ten minutes after the end of the fluid infusion; third, the effect on CO generated by a single fluid challenge is dissipated over a ten minutes period similarly in both groups.

Little is known about the pharmacodynamic effect of a fluid bolus of fluid. A pharmacokinetic/pharmacodynamic (PK/PD) model that could relate the PK behaviour with its observed therapeutic effect is complex, given the difficulties in measuring the "concentration" of the IV fluid bolus in blood samples. There are only a few studies published that describe the fluid PK in critically ill patients, as most describe the effects on less sick patients focusing on the changes in intra and extravascular volume with large amounts of fluid (25 ml·Kg^{-1})(26), or on fluid distribution across different fluid compartments (27) and also analysis of blood dilution as end-point of volume expansion (28). There are many studies (29-32) evaluating the haemodynamic effects of a fluid challenge between two time points (before and after the infusion). Recently, Nunes et al (33) reported an observational study in twenty patients with circulatory shock (14 septics) that received 500 ml of crystalloids over 30 minutes. The haemodynamics at 30 and 60 minutes after the end of fluid infusion were reported. As in our study, the authors observed that MAP and cardiac filling pressures were similar between responders and non-responders over time points and, along with CO, all haemodynamics decrease towards baseline 30 minutes after the fluid infusion. The rate of fluid infusion is lower than in our study and the period observed is longer, which make us question if the results are purely related to the fluid bolus in septic patients, who are normally quite dynamic. Glassford et al (11) performed a systematic review of the effect of a fluid challenge in

septic patients, observing again a rapid dissipation of the haemodynamic effects. Our findings are in accordance with these studies although, to our knowledge, this is the first study assessing the immediate effect of a fluid challenge on the circulation using a PD approach and its interaction with baseline values and CO response in a cohort of postoperative patients.

Overall effect of a fluid challenge: AUC

The global effect of a fluid challenge is similar between responders and non-responders for all tested haemodynamic variables except for CO and CVP. No difference was observed in MAP, which is in agreement with previous studies (29, 34-36). The arterial blood pressure is the result of the interaction between stroke volume and arterial elastance, and peripheral arterial pressure is also affected by pulse wave amplification (37, 38). AUC for CO was greater in responders, as expected, even when the maximal effect of the fluid challenge on CO does not happen immediately at the end of the fluid infusion, which is the most common value used to classify the groups (10). Actually, in 9 (37.5 %) fluid challenges from "non-responders", the maximal effect showed a further increase, achieving a change of CO greater than 10%. This may affect the results in other haemodynamic parameters, for example the CVP: while AUC is greater in non-responders none of the other outcomes achieved a level of evidence to support a clear difference between groups. The increase of CVP in non-responders is consistent with previous observations (39) and with the physiology of venous return: the flow is not increasing, the fluid is accumulated in the venous compartment, and the increase in CVP neutralises the increase of Pmsf (40). However, none of the other outcomes of CVP can be used to discriminate responders from non-responders.

The global effect of the fluid challenge on Pmsa was not different between groups, which is consistent with previous studies (22, 39). Pmsa is an analogue of Pmsf, which should increase after intravascular volume expansion, regardless of cardiac function.

Interestingly, those patients with higher values of MAP, Pmsa or CVP (non-responders) at baseline had smaller AUC respectively: this suggests that higher pressures do not necessarily mean lower compliance. Stress-relaxation in response to an increase in blood volume may increase vascular capacitance and reduce the global impact of the fluid challenge in the circulation, and this may be particularly evident when baseline pressures are already high enough.

Maximum effect size: d_{max}

The maximum effect size is similar between both groups for all the haemodynamics except for CO, which it is greater in responders. Even though the probability did not achieve clinical significance, our data suggest that HR decreases more in responders and CVP increases more in non-responders, although these estimated differences are very small. The probability that d_{max} in MAP was higher in non-responders is also close to a value of statistical relevance, however, the difference is so small (2.8 mmHg) that it would not be clinically relevant. Similar results were observed with CVP, the mean estimated difference between the two groups was -0.5 mmHg, which is again not clinically relevant. This emphasizes the importance of using flow-related variables to assess the response to a fluid challenge.

The correlations between dMax and baseline values suggest that the CO response plays as a moderator in the case of MAP, Pmsa and HR: the higher baseline levels of MAP, Pmsa and HR in responders, the smaller is the dMax observed, while in non-responders baseline does not affect the dMax values. In the case of HR, this suggests that a fluid challenge can reduce the HR only in responders that are actually tachycardic. For MAP

(and Pmsa) a possible explanation is the afterload effect on the left ventricle, so that the higher the MAP, the more difficult it is to drive the arterial pressure up, even when flow is still increasing.

Time to maximal effect

The time of maximal effect was different between groups for MAP, CO and Pmsa. Interestingly, the maximal effect on MAP and CO in responders was estimated at almost 2 and 1 minutes after the end of the fluid challenge respectively. During a bolus of IV fluids, part of the volume probably accumulates in the big veins and right atrium, and insofar as the end-diastolic ventricular volume is increasing, this volume is ejected into the systemic circulation. One potential cause of this delayed time is the presence of ventricular impairment or valve disease. Echocardiographic reports showed that most of our patients had normal ventricular size and function and only 6 – 7 % had valve disease (Table 1). Another explanation would be the activation of mechanisms implicated in the intrinsic inotropy of myocardial cells such as release of angiotensin II, endothelin, activation of the mineralocorticoid receptor, transactivation of the epidermal growth factor receptor and others (41). Even though this mechanism may take a little bit longer than 1 or 2 minutes to be fully activated, they might contribute to the increase of CO and MAP after the end of the fluid challenge.

In previous studies about fluid responsiveness, only 52 % of authors assessed the effect of the fluid challenge immediately after the fluid infusion, 21% did it between 1 and 10 minutes, while the rest of the authors reported the assessment time at 12, 30, or even 47 minutes after the fluid infusion (10). Glassford et al (11) reported that 10 of 19 studies assessed the haemodynamic effects immediately after the fluid infusion, 5 observed that effect 30 minutes after the fluid challenge and 3 studies reported the effect at 60 minutes time. Our findings suggest that, in order to avoid misclassifications regarding fluid

responsiveness, the effect of a fluid challenge on CO should be observed one minute after the end of the fluid infusion.

Interestingly, in those responders with higher MAP at baseline the maximal effect on MAP would be less intense but quicker, and in those patients with higher values of CO at baseline, the maximal effect on CO would be quicker. In non-responders, the maximum value can also be observed quicker in those patients with higher CVP at baseline, which make sense because a quicker rise of CVP would be expected when CVP at baseline is high in non-responders

Change from baseline after 10 minutes

After ten minutes all the haemodynamics variables tend to return to baseline similarly in both groups. Although there is a high probability that CVP remains slightly higher in non-responders, it did not achieve enough level of evidence, and conclusions about fluid responsiveness cannot be drawn based on the changes after 10 minutes. This rapid dissipation of the effect of the fluid challenge could have several explanations: a) the stress-relaxation mechanism as described by Guyton (13), which consist on a progressive stretching of the vessel wall that allows the intravascular pressure to return back to baseline over a period of minutes after a large increase in pressure in response to a rapid increase in intravascular volume. This mechanism may certainly explain the progressive decrease of CO, MAP, CVP and Pmsa. (b) Redistribution of the volume infused from the central circulation to the rest of the cardiovascular system, and particularly to the compliant veins (spleen, liver, big abdominal veins and cutaneous venous plexus) (c) Part of the volume infused may leak out of the circulation by either capillary leak or diuresis, although in ten minutes this explanation seems unlikely. (d) A decrease in vascular smooth muscle tone caused by sympathetic inhibition may decrease the Pmsf. Sympathetic nerves innervating the vasculature display a tonic activity that sets a

background level of vasoconstriction. Decreasing sympathetic outflow below this tonic

level causes vasodilation (42). It is known that in a short time scale (minutes – hours) the

autonomic nervous system adjusts the circulation in keeping with behaviour, emotions

and environment in order to meet the oxygen demand (43), so the influence of

sympathetic-related vasodilation cannot be totally excluded.

Clinical implications

The fluid challenge technique, at least as described in this study, should be

understood fundamentally as a diagnostic test for fluid responsiveness. This study

demonstrates that a single fluid challenge does not change CO over a long period of time.

Similar observations were made by Guyton(13), who used 30 – 50 ml/kg of three different

fluids infused in 2 – 4 minutes in 36 mongrel dogs. Likewise, Glassford et al (11) shows

that a fluid challenge in septic patients did not achieve any persisting haemodynamic

effect. Nunes et al (33) also reported a transitory effect using 500 mL infused over 30

minutes in septic patients. Importantly, stress-relaxation and redistribution of the

intravascular volume between stressed and non-stressed volume are physiological

mechanisms that allow adaptation to different intravascular volume status, so that they

take place in hypovolaemic, euvolaemic and hypervolaemic states (44, 45). Regardless

the baseline intravascular volume status, the transitory effect of a fluid challenge is also

determined by the dose of fluids given: in this study the average dose would be 3.3 ml/kg,

which is a lot less than the doses used in Guyton's experiments where a slower decay

effect was observed. Further research is needed to establish the minimal volume

required to perform a fluid challenge that significantly change the Pmsf and test the

circulation.

Our results suggests some important clinical implications: a) as previously

demonstrated (39) when a fluid challenge is performed using a rapid infusion rate and a

relatively "small" dose, its effect is sufficient to test if the patient is on the ascending part of the cardiac function curve , hence showing an increase in CO; b) the response to the fluid challenge is transitory, and as such also its clinical effect. Thus, our results emphasise that indications for further fluid therapy should not be made exclusively on the basis of the initial response to a fluid challenge as this may lead to fluid overload in some patients that transiently may increase their CO at every fluid challenge. Furthermore, fluid "unresponsiveness" should not be a clinical goal. Instead, optimal tissue perfusion should be the ultimate goal and must be evaluated before further fluids are given (44); c) the time when the response is assessed is an important factor when a clinician defines responders and non-responders. Likewise, when fluids are given with therapeutic purpose the assessment must take into account certain time for delayed compliance and volume redistribution, which could take at least ten minutes, depending on the dose given. It seems that the sustainability of haemodynamic changes depends on several factors such as the total volume of fluid given, the baseline haemodynamic values, the fluid redistribution rate and the baseline sympathetic tone. The effect of each particular factor will have to be evaluated in future studies.

The main difference between a fluid challenge and "fluid resuscitation" is a matter of dose: a fluid challenge can tell the clinician if a particular patient may increase CO by giving fluids. If the patient remains under-filled following the first fluid challenge, it seems logical that the effect on CO and Pmsa would tend to dissipate and the patient may require further fluid challenges. However, it must be emphasised that "fluid responsiveness" is not equivalent to "fluid requirement". In the context of fluid resuscitation, our results may suggest that continuous monitoring of CO and the use of additional interventions (vasoconstrictors) may be adequate in order to maintain CO, oxygen delivery and tissue perfusion over time. Similarly, protocols targeted to "maximisation" of the stroke volume might not necessarily represent an effective way of

maintaining a desired level of CO or MAP. The excess of volume in the circulation is compensated by redistribution between stress and non-stress volume, and in the worst cases, by a leak to interstitial space worsening tissue oxygenation. Further research is needed in order to find targets that can be used to guide fluid therapy regardless the fluid responsiveness status.

Limitations

There are several limitations in our study. First, the number of participants enrolled into the study is relatively small although comparable to other PD studies (46, 47).However, the total number of fluid challenges observed, which is the total source of variability analysed, represents a reasonable sample size. By using a multilevel statistical model, multiple measurements per subject can be observed taking into account two levels of variability (within subject and between subjects). This approach overcomes the limitation of analysing simple measurements per subject in small samples.

Secondly, many factors may affect the time-course effect of a fluid challenge on haemodynamics, so a reasonable question is if our findings can be extrapolated to a broader group of patients different from those in our sample. To answer this question we need to take into account a couple of considerations: first, the population of critically ill patients is very heterogeneous: vascular tone can vary considerably from post-operative to burned, from septics to trauma patients. To study the PD of a fluid challenge in such a heterogeneous population with a single mixed sample may result in invalid conclusions. We deliberately tried to select a relatively homogeneous group of participants (post-operative high risk surgical patients), taking into account the most relevant co-factors in order to make a fairly clear description of the fluids effect. Therefore, our study can only be generalised to this group of patients; the second consideration is related to the nature of the intervention: as mentioned earlier, the fluid challenge is performed around the

glove in many different ways. Our study included only patients that received 250 ml of crystalloids (Hartmann's solution) over five minutes. Logically, the type of fluid, the volume and the rate of infusion used could affect the haemodynamic response over time, so it would not be surprising to observe slightly different results with different techniques.

That said, our observations are in line not only with the cardiovascular physiology but also with the limited evidence available in other critically ill patients (11, 33). Although those studies did not observed PD outcomes, they also highlight the transitory haemodynamic effect of the fluids in both responders and non-responders. Further prospective studies are required to describe the PD pattern in other subgroups of critically ill patients.

Thirdly, the Pmsa is estimated using three measures: CVP, MAP and CO. Any inaccuracy in the measure of these variables has an impact on the value of Pmsa, in particular the CVP, so the results regarding Pmsa must be taken with caution. There are other methods described to measure Pmsf (22) in patients with intact circulation but they are technically difficult to implement for a continuous monitoring of Pmsf.

Fourth, although all efforts were made to exclude patients with established severe cardiac failure, it was not possible to obtain echocardiographic information for all the patients given the observational nature of this study. In addition, our patients were in a steady haemodynamic state: this is a condition required to obtain good quality data and to link the changes observed to the intervention performed. It is possible to observe different results in severely hypovolaemic or unstable patients. Finally, since this project was conducted using a clinical protocol, objective data reflecting to what extent the protocol was followed is not available.

Conclusions

The fluid challenge is an effective test for assessment of fluid responsiveness but its therapeutic effect on CO is dissipated in ten minutes. The maximal change on CO occurs at least over one minute after the end of the fluid infusion. The global effect of the fluid challenge on CVP is greater in non-responders, but not its change ten minutes after the fluid infusion.

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Reference Legends

Figure 1 Relationship between mean arterial pressure at baseline and dMax (maximal change from baseline) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).

Figure 2 Relationship between mean arterial pressure at baseline and tMax (time of maximal change from baseline) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).

Figure 3 Relationship between mean systemic filling pressure analogue (Pmsa) at baseline and AUC (area under the curve) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).

Figure 4 Relationship between mean systemic filling pressure analogue (Pmsa) at baseline and dMax (maximal change from baseline) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).

Figure 5 Relationship between central venous pressure (CVP) at baseline and tMax (time ofmaximal change from baseline) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).

Figure 6 Relationship between central venous pressure (CVP) at baseline and d10 (change from baseline at ten minutes time) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).

Figure 7 Relationship between heart rate (HR) at baseline and dMax (maximal change from baseline) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).

Figure 8 Relationship between heart rate (HR) at baseline and d10 (change from baseline at ten minutes time) observed and fitted and the interaction according to CO response: responders (Resp) and non-responders (Non Resp).