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Mallampati Classification, an Estimate of Upper Airway Anatomical Balance, Can Change Rapidly during Labor

THE Mallampati classification is a rough estimate of the tongue size relative to the oral cavity.[1] Although the single usage of the Mallampati classification has limited discriminative power for difficult tracheal intubation,[2] it is a simple, reproducible, and reliable preanesthetic airway assessment method when performed properly. In addition to difficult tracheal intubation, Mallampati class 3 or 4 is an independent predictor for difficulty of mask ventilation during anesthesia induction and presence of obstructive sleep apnea.[3,4] Increase of the Mallampati class during labor and delivery reported in this issue of ANESTHESIOLOGY[5] provides insight for exploring and understanding the mechanisms of difficulty in perioperative airway management of pregnant women, particularly during or immediately after labor. In the article, the authors thoroughly discuss the clinical implications of their findings on difficult tracheal intubation; therefore, I would like to assess their data focusing on perioperative upper airway obstruction of pregnant women.

Clinical Significance of Upper Airway Changes during Pregnancy and Labor

Kodali *et al.*[5] did not directly test the clinical significance of the increased Mallampati class because none of the women underwent general anesthesia; however, careful interpretation of their data reveals noticeable features of the upper airway structures in pregnant women. First, Mallampati class 3 and 4 seem to be more prevalent in parturients at the beginning of labor (28%) than in the general adult population (7–17%), suggesting that tongue volume increases even during normal pregnancy as previously reported.[6] Increased tongue volume presumably due to fluid retention during pregnancy may be partly responsible for increasing both prevalence of obstructive sleep-disordered breathing in pregnant women and incidence of difficult tracheal intubation in obstetric anesthesia.[7]

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More surprisingly and significantly, half of the parturients demonstrated Mallampati class 3 or 4 by the end of labor, predicting a further increase in difficulty of airway management during labor or immediately after delivery upon medical interventions such as general anesthesia. The risk of emergency cesarean delivery and surgery for postpartum hemorrhage is particularly high in obese parturients, presumably because of their higher incidence of maternal complications and fetal growth retardation.[7–9] Hood *et al.*[8] reported that 48% of laboring morbidly obese parturients required emergency cesarean delivery compared with 9% of control laboring parturients. Considering the high prevalence of obstructive sleep apnea in obese subjects and the growing problem of obesity among industrial countries, the finding of Kodali *et al.* is not trivial and carries particular importance to practitioners when anesthetizing obese parturients. In fact, a recent survey of anesthesia-related maternal deaths in Michigan identified obesity and African-American race as common characteristics of these cases.[10] Noticeably, there were no deaths during anesthesia induction, and five of eight anesthesia-related deaths resulted from hypoventilation or airway obstruction during emergence, endotracheal extubation, or recovery. Although safety of airway management during anesthesia induction seems to have greatly improved as a result of development of the airway algorithm and various intubation devices, an unsolved and significant problem in obstetric anesthesia is how to assess and manage the upper airway upon emergence and endotracheal extubation. The data of Kodali *et al.* suggest the labor is a potential risk factor for perioperative airway catastrophe in parturients in addition to obesity, craniofacial abnormalities, and sleep-disordered breathing. Pregnancy and labor are inevitable and physiologic processes for human beings that significantly burden the respiratory system by decreasing lung volume and thoracic compliance and narrowing the upper airway. Labor potentially makes some parturients more susceptible to pathologic upper airway narrowing.

Upper Airway Anatomical Imbalance in Parturients

The pharyngeal airway is a collapsible tube whose patency is precisely regulated by upper airway dilating muscles such as the genioglossus. Increase in the dilating muscle activity acts to maintain the narrowed pharyngeal airway during wakefulness in patients with obstructive

sleep apnea.[11] Similar neural mechanisms presumably compensate the progressive upper airway narrowing in parturients. Preservation of these neural regulatory mechanisms is, therefore, crucial for parturients with a high Mallampati class to maintain their breathing. Regional anesthetic techniques have only minimal influence on the neural mechanisms; however, the neural compensatory mechanisms become weaker during general anesthesia, sedation, and sleep with residual anesthetics. The pharyngeal airway patency entirely depends on its structural stability in parturients undergoing emergency cesarean delivery during general anesthesia.

Structurally, the pharyngeal airway is surrounded by soft tissues such as the tongue and soft palate, which are enclosed by bony structures such as the mandible and spine. Size of the airway space is determined by the balance between the bony enclosure size and soft tissue volume (anatomical balance) when pharyngeal muscles are inactivated by general anesthetics and muscle relaxants.[12] Pharyngeal edema, presumably due to fluid retention during pregnancy, and pharyngeal swelling acutely developed during labor increase the soft tissue volume surrounding the airway, narrowing the pharyngeal airway in parturients. Recent extensive research on the pathophysiology of upper airway obstruction revealed a significant role of the lung volume reduction in pharyngeal narrowing. Tagaito *et al.*[13] demonstrated that lung volume dependence of pharyngeal airway patency is more pronounced in obese patients. Accordingly, obese parturients, a high-risk group for perioperative airway catastrophe, are prone to develop progressively narrower pharyngeal airways due to increase of soft tissue volume surrounding the pharyngeal airway and decrease of lung volume during pregnancy. Lung volume reduction during general anesthesia is known to be more prominent and prolonged in obese patients. General anesthesia for emergency cesarean delivery in obese parturients during or immediately after labor may tend to exaggerate upper airway swelling and lung volume dependence, in addition to impairment of neural compensatory mechanisms, and is, therefore, a worst-case scenario for upper airway maintenance. Application of positive end expiratory pressure during anesthesia and full consciousness at endotracheal extubation are strongly recommended for these patients.

Mallampati Classification for Assessment of Upper Airway Anatomical Balance

Kodali *et al.* demonstrated a decrease in upper airway volume of approximately 10 ml during labor and delivery. Although they did not simultaneously assess changes in Mallampati class in this group of parturients, it is of interest how much reduction of the upper airway volume, *i.e.*, how much increases in the tongue volume, leads to a 1-point increase in Mallampati class. Assuming similar changes of the Mallampati class in both study groups, *e.g.*, a 26-point increase of the Mallampati class in 61 subjects leads to a 10-ml reduction of upper airway volume on average, it can be roughly estimated that a 1-point increase of the Mallampati class approximately corresponds to a 20-ml increase of the tongue volume in women with Mallampati class 3 or 4 before labor. Upper airway volume differed between patients with and without difficult tracheal intubation by 30–40 ml.[14] Tongue volume was significantly larger in patients with obstructive sleep apnea, by approximately 20–25 ml, than in nonapneic persons.[15] For every 1-point increase of the Mallampati class, the relative risk of obstructive sleep apnea doubles and apnea hypopnea index increases by 5 h^{-1}.[4] Accordingly, a 20-ml increase of the tongue volume during labor potentially results in difficult tracheal intubation and upper airway obstruction under influence of general anesthetics and sedatives.

The Mallampati classification allows us to instantaneously identify such small but significant increases in the tongue volume at the bedside without using sophisticated apparatuses. The Mallampati classification originated in our specialty, and recently, clinicians and researchers in other specialties have recognized its usefulness for assessment of upper airway anatomical balance. We anesthesiologists should be proud of the Mallampati classification and are encouraged to use this classification to assess the upper airway anatomical balance with it before every general anesthesia induction. The article by Kodali *et al.*[5] reminds us that the Mallampati classification is not static, but can change over hours with processes such as labor, and we should assess it just before instrumentation, rather than relying on an assessment even a few hours earlier.

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Impact of Analgesia on Bone Fracture Healing

BONE fractures are painful and, in general, temporarily disabling injuries that occur after trauma or as an end result of various pathologic conditions, such as osteoporosis or bone invading cancer (pathologic fracture). It is common practice for acute pain services to be heavily involved in the treatment of orthopedic patients, and although it is usually understood that fractures are very painful, few studies have directly attempted to measure or define the nature of the pain after a fracture. In addition, controversy exists regarding the effects of analgesics, including opioids and nonsteroidal antiinflammatory drugs, on skeletal tissue healing. In this issue of ANESTHESIOLOGY, reports from Freeman *et al.*[1] and Minville *et al.*[2] show that commonly used fracture healing models also can be used to assess pain quantitatively and therefore to assess analgesic efficacy. Because bone is a highly innervated tissue, these models also can be applied to define the mechanism of pain transmission after fracture. Furthermore, and of even greater clinical importance, these fracture pain models lend themselves to studying the effects of pharmacologic interventions on bone healing.

Bone fractures are treated by restoring the anatomy of the broken bone (reduction) and immobilizing the bone pieces (fixation) while regeneration proceeds. Commonly, fracture fixation is done by casting the broken bone. This also can be achieved surgically by use of intramedullary rods or external fixators that use percutaneous pins or rods to hold the bone fragments in correct anatomical alignment. In these cases, the fracture site is not significantly disturbed and the fractures heal by bone regeneration. Initially, the fracture causes localized tissue hypoxia and hematoma formation and is soon followed by a robust inflammatory response. Next, mesenchymal cells migrate and proliferate at the fracture site to form a callus. Concomitantly, osteoblasts in the periosteum near the fracture site begin to proliferate. At the interface with the periosteal osteoblasts, the mesenchymal cells differentiate into chondrocytes and elaborate a cartilage matrix. Eventually, the chondrocytes undergo hypertrophy and mineralize the cartilage matrix, which then acts as a substratum for osteoblast bone formation. This process is reiterated from the periphery of the fracture site toward the center until the fracture is bridged with newly formed bone and is dependent on angiogenesis. Subsequently, the bony callus is remodeled to restore the mechanical properties of the bone. This is the normal endochondral ossification pathway of fracture healing and is often referred to as secondary fracture healing. In contrast, primary fracture healing occurs only after surgical fixation of the fracture in which the fracture callus is removed, and the bone ends are closely abutted and rigidly fixed in place, usually with a plate. In this case, the fractures heal slowly *via* normal bone remodeling mechanisms while the metal plate stabilizes the fracture and provides for any weight-bearing functions.

Pharmacologic treatments that can affect the molecular and cellular processes of bone regeneration can have a significant impact on healing. For example, fracture healing is severely impaired in rats treated with TNP-470, an antiangiogenic compound.[3] More important in terms of pain management, nonsteroidal antiinflammatory drugs and cyclooxygenase-2 inhibitors, such as celecoxib, impair fracture healing in animal models.[4,5] Eight weeks after fracture, twice the normal bridging time in rats, femur fracture healing had failed in approximately one third of female rats treated with celecoxib (4 mg/kg daily) for 5 days after fracture. However, celecoxib therapy before fracture or celecoxib therapy initiated 2 weeks after fracture had no significant effect on healing in rats. Limited retrospective data also indicate that these effects may translate to humans.[6] Among acetabular fracture patients treated with indomethacin or localized radiation to prevent heterotopic ossification, 29% of those patients treated with indomethacin experienced a non-

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union in another long bone fracture as compared with 7% of the patients treated with localized radiation.

Similarly, opioids are most frequently used to treat severe pain caused by metastatic bone cancer (*e.g.*, breast and prostate cancer) but do have a variety of nonskeletal (and potentially skeletal) side effects that could inhibit bone healing. Opioid side effects such as sedation, clouding of mental status, or cognitive impairment can reduce mobility, resulting in loss of bone and muscle mass. This is particular disturbing in the elderly population, where hip fractures have a 20% mortality rate within the first year and bed rest needs to be as short as possible to minimize inactivity-induced bone and muscle loss as well as pulmonary complications.

Opioids also may have direct, detrimental effect on bone healing. Recently, it was shown that analgesic therapy provided by morphine accelerated sarcoma-induced bone destruction and doubled the incidence of spontaneous fracture in mice.[7] It is not known whether the findings in this model of osteolytic sarcoma will generalize to other cancers or opioids. For example, tramadol did not significantly inhibit human osteoblast activity *in vitro*.[8] Undoubtedly, these data suggest a need for increased research in this field. Identifying new analgesic therapies that will enable early physical therapy and that do not impair healing or cognitive function may provide real lifesaving benefits for many patients.

Despite this obvious need, the pain response after fracture has not been studied previously in detail in any animal model. Minville *et al.*[2] and Freeman *et al.*[1] demonstrate that common fracture models used in mice and rats to study fracture healing can be applied to studying the mechanisms of pain transmission and the extent to which analgesic therapies can ameliorate pain. Using a closed tibia fracture model in mice, Minville *et al.* assessed subjective pain and measured mechanical and thermal nociception in the affected limbs using von Frey hairs and a modified hot-plate test, respectively. As expected, pain scores were highest in the days immediately after fracture and began to decline approximately 5 days after fracture, which likely corresponds to resolution of inflammation after the fracture. Morphine or ketoprofen treatment significantly reduced pain. Similarly, using a closed femur fracture model in rats, Freeman *et al.* were able to show that behavioral indications of pain after fracture (guarding behavior and flinching) closely followed the voluntary amount of weight displaced by the rat on the fractured limb. As might be expected, these indicators of pain were highest in the first days after fracture and began to recede after approximately 7 days. Morphine treatment dose-dependently decreased measures of pain. However, the investigators noted that the highest morphine dose tested (10 mg/kg) led to lethargy in the rats, which impeded the researchers' ability to measure improvements in weight bearing with this dose.

The animal models described in this issue of ANESTHESIOLOGY by Freeman *et al.*[1] and Minville *et al.*[2] seem to be posed ideally to advance the science in this field further and foster collaborations among orthopedic surgeons and anesthesiologists. An advantage of the rat fracture model seems to be that the fracture production is easily accomplished in the larger rats and, overall, more knowledge has been gained in various pain models in rats, but the mouse fracture model affords the possibility of using genetically modified animals. Although neither study ultimately assessed the effects of morphine or ketoprofen on fracture healing outcomes (yet), these studies demonstrate that established fracture models can be used to measure the effects of different therapies on pain and bone healing, thereby providing us with a tool for further improvement of the surgical outcome of our orthopedic patients.

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Understanding Methadone Metabolism

A Foundation for Safer Use

METHADONE has become one of the darling drugs of the pain management community and is used in a variety of situations. Far from being restricted to use in addiction treatment centers, the drug is now frequently prescribed for the management of pain related to malignancies and even more frequently for nonmalignant types of pain. One review found the number of population-adjusted methadone prescriptions increased 727% from 1997 to 2004, and that this increase was due in general to an increase in prescriptions for pain.[1] The readers of this journal may also be familiar with the drug's use perioperatively when a sustained base of analgesia is desired as part of the anesthetic plan or perioperative pain management scheme. Indeed, this drug has many properties making it useful in these settings. It is potent and has high efficacy, excellent absorption from the gastrointestinal tract, no known active metabolites, very low cost, and both oral and intravenous formulations. The weak noncompetitive *N*-methyl-D-aspartate receptor antagonism possessed by methadone may further enhance the analgesia of the drug. The name of one of the first formulations, Dolophine, in fact, comes from *dolor* ("pain") and *fin* ("end"). What could possibly be the problem with this pain-ending drug? As explained by Totah *et al.* in the introduction to their article, "Role of CYP2B6 in Stereoselective Human Methadone Metabolism," the drug has an exceedingly variable but generally slow hepatic clearance.[2] The variable but usually very long half-life governed by this slow clearance has raised serious concerns over this drug's safety.

The chief serious hazard associated with this opioid when used for pain management is respiratory depression. What makes this particular opioid more problematic than most is that accumulation to steady state can take a week or more. This slow approach to steady state levels brings with it the possibility that side effects can also appear slowly. In the majority of cases, the drug is administered to outpatients; therefore, the patients are unmonitored as the drug accumulates. Specific populations such as the elderly or those prone to abuse of medications may be at particular risk for delayed respiratory depression caused by methadone. Data strongly support the notion that methadone's slow accumulation can have lethal consequences. For example, Sims *et al.*[1] found that the number of methadone deaths increased 1,770% from 1997 to 2003. Appalachian states once hard hit by OxyContin abuse now seem to be particularly hard hit by methadone-related problems as well.[3] Many other articles have commented on methadone's particular problem with slow accumulation and toxicity and have generally recommended a "start low, go slow" approach.[4,5] It is quite rational, then, to focus studies on methadone's metabolism to predict who might be particularly prone to methadone-related toxicity, and under what circumstances toxicity might be seen.

Investigators have for some time been interested in methadone's hepatic clearance. The cytochrome P450 (CYP) enzyme system has long been known to play a major role in first phase methadone metabolism. The more important unanswered question has been, "Which isoform?" The hepatic CYP enzyme family has approximately 50 members expressed in humans. Many are concentrated in the liver, although CYP-mediated metabolism occurs in many other organs, including the gut, lungs, and brain. Far from drug specific, individual drugs can be substrates for multiple CYPs. Making matters more complex, the predominant metabolizing isoform may depend on which isomer of a racemic mixture of drug is being followed. With respect to methadone metabolism, several isoforms including the CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 have been implicated. This might prompt the reader to ask what the value of further study would be if there are so many enzymes known to be involved. The goal of the studies of Totah *et al.* was therefore not necessarily to ask which enzymes *are able* under some set of conditions to metabolize the drug, but to assess which isoforms *really do* dominate human metabolism, thus providing us a more clinically useful metabolic characterization. The answer in this case was not entirely expected. Using a series of pharmacologic tools and a rigorous analytical strategy, the authors were able to implicate the CYP2B6 enzyme as that responsible for the bulk of methadone metabolism in humans, particularly the *S*-methadone isomer. Therefore, not only overall plasma levels but also the ratio of plasma methadone isomers is controlled by CYP2B6.[2]

Of special note are genetic factors impacting metabolism through the CYP enzyme system, particularly the CYP2B6 and CYP3A4 isoforms the authors conclude are likely important for hepatic methadone metabolism.

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Both genes are polymorphic in humans, and the polymorphic nature of both genes has been demonstrated to affect the rates or clearance, production of metabolites, and probability of reaching clinical endpoints for various drugs, including methadone. Crettol *et al.*,[6] for example, attempted to associate CYP2B6, CYP2C9, and CYP2C19 polymorphisms with methadone plasma levels in 209 patients in methadone maintenance with positive associations found for only the CYP2B6 variants. The predominant effects were on *S*-methadone levels, which is consistent with the findings of Totah *et al.*[2] In a follow-up study by the same group involving 245 patients in methadone maintenance, these authors reproduced their initial genetic associations with *S*-methadone metabolism and CYP2B6 variants, although CYP3A4 activity was also linked to plasma methadone levels in this study.[7] Zanger *et al.*[8] have demonstrated the CYP2B6 enzyme to be highly polymorphic, and more detailed studies will be required to determine which haplotypes of the more than 100 single nucleotide polymorphisms are associated with altered enzymatic activity. Other studies have associated CYP3A4 activity with total methadone plasma concentrations, and possibly with the rare methadone-related complication of prolonged QT interval and torsade de pointes.[9] The genetics and impact on disease have been far more carefully studied for CYP3A4, and many publications are dedicated to variants of the gene for this enzyme as they impact drug disposition of opioids, benzodiazepines, chemotherapeutics, and other drugs as well as susceptibility to diseases, particularly cancers.

The field of medicine is experiencing growing interest toward individualizing medical care. This approach to therapy takes into account multiple aspects of a patient's makeup when selecting and implementing treatments. Determinants of drug metabolism figure prominently in this approach. Methadone is an effective treatment option for many patients but has a clear record of at least one severe complication, respiratory arrest. Better understanding the process of clearance of this drug stands a reasonable chance of improving the safety and outcomes of methadone use in patients, particularly if genetic or other types of profiling could be shown to accurately predict plasma drug levels. Understanding the control of individual isomer levels for racemic drugs in which the isomers have different pharmacologic properties needs to be taken into account. With their expertise in the investigation of drug disposition, laboratories like those of Totah *et al.*[2] are in excellent position to help anesthesiology and its related fields take a lead position in this emerging area of medicine.

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Preemptive Antihyperalgesia to Improve Preemptive Analgesia

BASED on the rationale that gabapentin effectively treats neuropathic pain and that there are many similarities in mechanisms between neuropathic pain and hyperalgesia from opioid administration,[1] Van Elstraete *et al.*[2] tested whether gabapentin could also reduce hyperalgesia induced by a short-term use of fentanyl in rats, and reported in this issue of ANESTHESIOLOGY that it was effective, although no analgesic effect *per se* was observed. These observations agree with a recent study in human volunteers showing that pregabalin, a gabapentin analog, has no effect on electrically evoked pain but significantly reduces the areas of punctuate mechanical hyperalgesia.[3] Numerous experimental and clinical studies suggest that gabapentin is unlikely to be a conventional analgesic and may have a selective effect on pain processes involv-

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ing central sensitization, such as that which occurs with opioid exposure. This result highlights new ways of thinking to improve the management of postoperative pain.

Although opioids are well recognized as unsurpassed analgesics for relieving moderate to severe pain, clinical studies have reported for more than a century that hyperresponsiveness to noxious stimuli is the most common symptom of withdrawal after prolonged opioid administration. More recently, the paradoxical phenomenon of opioid-induced hyperalgesia (OIH) has been described to develop rapidly after a single opioid exposure in animals, human volunteers, and surgical patients.[4] OIH is now recognized to reflect a sustained sensitization of the nervous system in which excitatory amino-acid neurotransmitter systems play a critical role, especially *via* *N*-methyl-D-aspartate (NMDA) receptors. From a medical viewpoint, abnormal persistence of excitatory neuroplasticity is now considered to be a major, if largely unrecognized, candidate mechanism for the development of chronic pain.[5] One hypothesis is that the administration of large doses of opioids, as used for surgery in humans, increases the risk to induce not only early exaggerated postoperative pain, but also the development of postoperative chronic pain. This does not mean that opioid use must be eliminated for surgical analgesia in humans (certainly not!) but provides yet another rationale to reduce opioid dose to avoid unidentified and then unevaluated long-lasting deleterious effects on pain sensitivity. Moreover, therapies that can oppose early postoperative hyperalgesia and reduce postoperative opioid consumption should be developed, because these are perhaps linked phenomena. Several points should be considered.

From a mechanistic viewpoint, experiments in animals and human volunteers show that drugs that affect NMDA receptor functioning directly, such as NMDA receptor antagonists like ketamine, memantine, dextromethorphan, magnesium, or nitrous oxide, or indirectly by reducing the spinal release of excitatory amino acid neurotransmitters, such as the cyclooxygenase inhibitors, prevent OIH by inhibiting an overactivation of pronociceptive systems and antiopioid systems that directly oppose the analgesic effects of opioids.[5] Gabapentin acts *via* different mechanisms than NMDA receptor antagonists or cyclooxygenase inhibitors because it presynaptically binds to the $\alpha_2\delta_1$ subunit of voltage-gated calcium channel ($Ca_v\alpha_2\delta_1$). Elevated $Ca_v\alpha_2\delta_1$ at the spinal cord level has been proposed to contribute to pain hypersensitivity in neuropathic pain models.[6] Interestingly, gabapentin effects on voltage-gated calcium channel currents are minimal in wild-type mice as compared with significant inhibition in transgenic mice that constitutively overexpress $Ca_v\alpha_2\delta_1$ in neuronal tissues.[7] Voltage-gated calcium channel current blockade by pregabalin leads to a decrease of release of the excitatory neurotransmitters substance P and glutamate, which are themselves known to be involved in pain hypersensitivity. One hypothesis (which does not exclude other mechanisms) is that gabapentin indirectly prevents OIH *via* inhibition of overactivation of NMDA receptors like other antihyperalgesic drugs. However, an up-regulation of neuronal $Ca_v\alpha_2\delta_1$ in this OIH experimental model has not been investigated. When this information is taken together, it is conceivable indirect modulation of NMDA receptor functioning by gabapentin is more useful clinically than direct receptor blockade by NMDA receptor antagonists for opposing OIH because side effects are more limited with the former approach. This is also supported by our recent observation that a polyamine-deficient diet, which negatively modulates overactivation of NMDA receptors *via* an NMDA allosteric polyamine site, is an efficacious strategy devoid of any noticeable side effects to relieve pain hypersensitivity.[8]

From a therapeutic viewpoint, these data could lead us to reevaluate preemptive analgesia. Preemptive analgesia is defined as a treatment initiated before the surgical procedure to prevent pain and sensitization. Numerous clinical studies indicate that the level of preoperative pain is correlated with the development of early postoperative pain and with the subsequent development of chronic pain, which may persist for months or years after surgery. Indeed, even though the best way to prevent pain sensitization might be to block completely any pain signal originating from the surgical wound from the time of incision (or opioid administration) until final wound healing, it is noteworthy that analgesic drugs used for surgery have different pharmacologic profiles. Therefore, opioids, though potent analgesics, are typically "false friends" for preemptive analgesia because they also induce delayed and long-lasting pain hypersensitivity. As indicated by Eisenach[9] in an Editorial View in this journal, opioids might induce "Preemptive hyperalgesia, not analgesia." Indeed, the study by Van Elstraete *et al.*[2] and related OIH studies suggest that analgesia is necessary but not sufficient to develop good preemptive analgesia and that a preemptive antihyperalgesic is also needed. Among available therapies, the early administration of antihyperalgesic agents that are not antinociceptive *per se*, such as gabapentin; polyamine-deficient diet; or analgesics with specific antihyperalgesic properties, such as buprenorphine, nitrous oxide, cyclooxygenase inhibitors, or nefopam might be fruitful strategies for improving preemptive analgesia. These agents may be also preferentially used during the postoperative period to reinforce this antihyperalgesic strategy.

Finally, from a medical viewpoint, patients with high intensity of early postoperative pain are known to have a higher risk of developing a chronic pain state.[5] Opioids might reinforce this deleterious phenomenon. Experimental[10] and clinical studies[11] indicate that preoperative and perioperative therapies that are efficacious to prevent early exaggerated postoperative hyperalgesia also

oppose long-lasting pain hypersensitivity, *i.e.*, pain vulnerability. This phenomenon, which is paradoxically facilitated by opioids, has been referred to as a "latent pain sensitization"[10] because it may aggravate preexisting pain that could have gone unnoticed in the absence of subsequent nociceptive inputs. Gabapentinoids have demonstrated promising antihyperalgesic potential in a number of clinical trials of early postoperative pain. However, the ability of gabapentin to prevent the development of postoperative chronic pain must be evaluated in combination with different classes of "traditional" analgesics. Indeed, we must reevaluate analgesics, especially opioids, independently of their own analgesic potencies because OIH studies indicate that certain opioids, especially fentanyl and remifentanil, induce hyperalgesic effects, whereas others, such as buprenorphine or methadone, exert a lasting antihyperalgesic effect.[12] Attempting to combine preemptive analgesia with preemptive antihyperalgesia could be a fascinating challenge for modern anesthesiology because chronic pain occurs in 10–50% of patients after surgery, especially when it is associated with nerve injury. Other therapies, such as continuous local anesthetic wound infusion or systemic antiinflammatory corticosteroid therapy, might reinforce preemptive antihyperalgesia. Although the possibilities are exciting, application of such therapeutic strategies will also need to apply Hippocrates' dictum, *primum non nocere*—first, do no harm.

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Circulating Progenitors in Lung Injury

A Novel Therapy for Acute Respiratory Distress Syndrome?

ACUTE respiratory distress syndrome (ARDS), and its less severe form, acute lung injury (ALI), are common disorders in patients requiring critical care, occurring secondary to a variety of injuries such as aspiration, sepsis, and trauma. Mortality from ARDS approximates 40%, and no specific medical therapies exist despite years of well-conducted clinical trials.[1] This compels investigators to explore novel therapies aimed at treating this disorder. In this issue of ANESTHESIOLOGY, Lam *et al.*[2] present data suggesting that an infusion of autologous endothelial progenitor cells (EPCs) ameliorates experimentally induced lung injury. Their findings highlight the potential of cell-based therapies for treatment of ALI and ARDS.

This Editorial View accompanies the following article: Lam C-F, Liu Y-C, Hsu J-K, Yeh P-A, Su T-Y, Huang C-C, Lin M-W, Wu P-C, Chang P-J, Tsai Y-C: Autologous transplantation of endothelial progenitor cells attenuates acute lung injury in rabbits. ANESTHESIOLOGY 2008; 108:392–401.

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Diffuse endothelial damage is a hallmark of ALI. In its earliest phases, endothelial cells become edematous and gaps develop between normally tightly adjacent cells. These changes expose the basement membrane, contribute to the development of hyaline membranes, and result in the filling of alveolar spaces with proteinaceous pulmonary edema fluid.[3] Potential therapies targeted at repair or limiting of endothelial damage in ALI have not been thoroughly explored, although it seems reasonable that such therapy might benefit patients with ALI. The oleic acid lung injury model used in this study seems to be a logical surrogate to test the effect of cell-based therapy directed at endothelium damaged in ALI.

The initial observation of circulating progenitor cells with endothelial properties was met with enthusiasm

because it held the promise of cell-based therapies for a myriad of vascular diseases.[4] Nevertheless, isolation and characterization of EPCs has proven difficult. Their numbers in the circulation of both healthy individuals (as well as in animals) is exceedingly low.[5] Also, no consensus exists on the type or types of cell surface marker(s) for EPCs. There is some agreement that the cell surface antigens CD133 and vascular-endothelial growth factor receptor 2, along with LDL uptake and lectin binding, may be used to identify these cells.[6] In addition, culture in endothelial-specific medium has been shown to promote the growth of cells with endothelial progenitor properties.[7]

An important recent observation relevant to EPC investigations is the identification of two EPC subtypes with potentially different roles in endothelial repair.[8,9] These two disparate cell types are obtained by culturing peripheral blood mononuclear cells and examining certain properties *in vitro*. Peripheral blood mononuclear cells that grow into colony-forming units on fibronectin early (approximately 7 days) are termed *early EPCs*. They possess cell surface antigens consistent with hematopoietic progenitors (*i.e.*, CD34), along with endothelial-specific markers (*i.e.*, CD31) and markers of monocytic lineage (*i.e.*, CD14), among others. They have less potential to form true endothelium in tube-forming assays in three-dimensional cultures either in isolation or in coculture with human umbilical vein endothelial cells. However, early EPCs have been reported to secrete significant amounts proangiogenic growth factors.[10] In contrast to this, late or outgrowth endothelial progenitors appear after 2 or more weeks in culture and do not secrete these factors to a measurable extent. Late EPCs do share some markers in common with early EPCs, such as CD31, but do not possess hematopoietic surface antigens. These cells may be more operational in replacing damaged or destroyed endothelium, because *in vitro*, they will readily form endothelioid tubes and functional blood vessels in animal models. Therefore, it may be that each of these cell types plays a unique role in proper endothelial repair.[11]

Before oleic acid–induced lung injury, Lam *et al.* isolated early EPCs by culturing peripheral blood mononuclear cells for 1 week. Cultured cells were then collected and injected into lung-injured and uninjured control animals. When the animals were killed 48 h later, fluorescent-labeled EPCs were detected in pulmonary arterioles of animals injected with EPCs. Western blotting analysis performed on pulmonary arterial blood of these animals revealed that inducible nitric oxide synthase expression was suppressed in animals that had received EPCs. In addition, animals subjected to lung injury who received EPCs had a decreased wet-to-dry weight ratio, a decrease in hyaline membrane formation, decreased hemorrhage, and a lower percentage of neutrophils present within the lung. Although not endothelial specific, these findings suggest that EPC infusion had an effect on preservation of alveolar–capillary barrier integrity.

Why did early EPC infusion seem to be beneficial in lung injury? One answer may be these cells' ability to secrete antioxidants and thereby ameliorate the highly oxidized milieu in the lung observed in ALI, reflected in the animal model as high inducible nitric oxide synthase expression.[12] These authors performed additional experiments *in vitro* to assess the antioxidant capacity of human early EPCs compared with the antioxidant capacity of human umbilical vein endothelial cells. Expression of the antioxidants manganese superoxide dismutase and heme oxygenase 1 were greater in early EPCs compared with the more mature human umbilical vein endothelial cells. These findings suggest a potential mechanism whereby infusion of early EPCs acts to normalize the oxidative milieu of the injured lung and potentially lay the groundwork for adequate lung repair and normalization of cellular function.

This study by Lam *et al.* provides proof of the concept that infusion of early EPCs may be beneficial in ARDS and ALI. However, it is not without certain limitations. First, although injected cells EPCs appeared in the pulmonary vasculature, they were not observed elsewhere in the lung, such as within the alveoli, where lung repair is most needed. Also, the degree of EPC engraftment (or at least association) was not quantitated, although this has proven difficult for other investigators to assess as well. Therefore, it is unclear exactly what role EPCs have in preserving or restoring lung architecture, although lungs of animals given EPCs appeared significantly more normal after injury. Given the degree of lung damage elicited by oleic acid, the relatively small number of EPCs infused, and the short time period between EPC infusion and animal death, it is less likely that EPCs themselves played a significant structural role in repair of damaged endothelium. However, a paracrine role for these cells seems more plausible. Another issue raised by the authors is the relevance of an autologous cell infusion model for ALI, where it would be impossible to collect cells from a patient before disease onset to use later on in illness. A more feasible possibility might be to use therapies such as granulocyte–macrophage colony-stimulating factor that enhance endogenous EPC release and could be used after lung injury has occurred. Further clarification of the role of these therapies could be expected to move to clinical trials more quickly than cell-based therapies, because the latter will likely be subject to intense regulatory scrutiny.

Endothelial progenitor cells have proven enigmatic to study. However, despite our lack of clearly understanding their purpose and function, animal models of lung injury such as that detailed in this study and others indicate beneficial effects of EPCs in terms of preservation of lung architecture after injury.[13] Nonetheless, additional investigations are clearly required to determine whether ben-

efits of this type of therapy are clinically significant and sustained and to ensure that there are no unanticipated adverse effects. Although cell-based therapies for lung injury are still a faraway goal, studies such as this by Lam *et al.* provide important information that will undoubtedly aid in the development of novel therapies for ALI and ARDS.

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