Anesthesiology 2008; 108:335

In Reply:—We are grateful to Dr. Tobinick for his clinical work evaluating etanercept for spinal pain, and his astute and prescient comments regarding our past and future endeavors.[1] First, we would like to point out that intradiscal tumor necrosis factor-α administration to relieve radicular pain is not quite analogous to the intradiscal injection of corticosteroid, which has been shown in previous studies to be no more effective than placebo for this condition.[2,3] Although inflammatory cytokines released from a degenerative disc might be the source of a painful, chemically irritated nerve root,[4–6] the disc itself is not the primary site of inflammation. Therefore, it is not surprising that intradiscal steroids are ineffective for lumbosacral radiculopathy. For predominantly axial low back pain presumed secondary to internal disc disruption, there is no scientific basis to suppose that the epidural injection of tumor necrosis factor-α inhibitors might be effective.

In contrast, the "mechanistic-based treatment of pain" paradigm advocates identifying the principal pain generator (*i.e.*, high concentrations of tumor necrosis factor α expelled from a degenerated disc) and treating it with target-specific medications (*i.e.*, tumor necrosis factor-α inhibitors).[7] In this context, injecting etanercept intradiscally can be viewed as a logical extension of this theory.

Second and perhaps more importantly, Dr. Tobinick seems to have overlooked the possibility that our intradiscal study was never intended to be the decisive word on the subject. Rather, our main objectives in undertaking this endeavor were to establish safety (hence our low, logarithmically increasing doses) in this setting and to determine dose ranges for the more definitive and auspicious epidural study he alluded to. The risk:benefit ratio is considerably higher for the epidural administration of etanercept in radiculopathy, a condition for which effective treatments are available, than it is for refractory low back pain patients already scheduled to undergo discography in a last-ditch effort to determine eligibility for either experimental intradiscal procedures or spine surgery. In addition, we have previously demonstrated that a significant portion of intradiscal injectate extravasates into the epidural space in patients with degenerative disc disease.[8] This suggests that the poor response of our patients may better reflect their long duration of pain (inflammatory cytokines play a more prominent role in acute pain than chronic pain) and multiple previous treatment failures, rather than the intradiscal route of administration.

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References

1. Cohen SP, Wenzell D, Hurley RW, Kurihara C, Buckenmaier CC III, Griffith S, Larkin TM, Dahl E, Morlando BJ: A double-blind, placebo-controlled, dose–response pilot study evaluating intradiscal etanercept in patients with chronic discogenic low back pain or lumbosacral radiculopathy. ANESTHESIOLOGY 2007; 107:99–105
2. Khot A, Bowditch M, Powell J, Sharp D: The use of intradiscal steroid therapy for lumbar spinal discogenic pain: A randomized controlled trial. Spine 2004; 29:833–6
3. Simmons JW, McMillin JN, Emery SF, Kimmich SJ: Intradiscal steroids: A prospective double-blind clinical trial. Spine 1992; 17 (suppl):S172–5
4. Ozaktay AC, Cavanaugh JM, Asik I, DeLeo JA, Weinstein JA: Dorsal root sensitivity to interleukin-1 beta, interleukin-6 and tumor necrosis factor in rats. Eur Spine J 2002; 11:467–75
5. Olmarker K, Larsson K: Tumor necrosis factor (alpha) and nucleus-pulposus-induced nerve root injury. Spine 1998; 23:2538–44
6. Igarashi T, Kikuchi S, Shubayev V, Myers RR: Exogenous tumor necrosis factor-alpha mimics nucleus pulposus-induced neuropathology. Spine 2000; 25: 2975–80
7. Woolf CJ, American College of Physicians, American Physiological Society: Pain: Moving from symptom control toward mechanism-specific pharmacologic management. Ann Intern Med 2004; 140:441–51
8. Cohen SP, Oberfell R, Larkin T, Fant G, Stojanovic M: Does needle insertion site affect discography results? A retrospective analysis. Spine 2002; 27:2279–83

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Anesthesiology 2008; 108:335–6

Perioperative Protective Ventilatory Strategies in Patients without Acute Lung Injuries

To the Editor:—We enjoyed reading the recent editorial and review article about optimal tidal volume (V_T) in patients without acute lung injury.[1,2] Overstretching healthy lungs with "traditional" V_T in the range of 10–15 ml/kg predicted body weight has been shown to trigger inflammatory and procoagulant alveolar responses. Furthermore, synergism rather than additivity between ventilator-induced alveolar stress and other injurious pulmonary factors (sepsis, ischemia–reperfusion, hypoxia–reoxygenation, major trauma and surgery) has been incriminated in damaging the alveolocapillary barrier. Ultimately, a multiple hit concept has emerged to explain the pathophysiologic mechanisms of acute lung injury.

We fully agree that protective ventilatory strategies (V_T of 6 ml/kg predicted body weight, inspiratory plateau pressure <20 cm H_2O, positive end-expiratory pressure [PEEP] levels >5 cm H_2O) currently applied in the intensive care unit should also be adopted to manage surgical patients with "vulnerable" lungs (*e.g.*, ongoing inflammatory/infectious disease, lung resection, major trauma and surgery). Unfortunately, in the majority of surgical patients with "healthy" lungs and no acute lung injury risk factors, the proposed ventilatory guidelines (V_T <10 ml/kg predicted body weight, inspiratory plateau pressure <20 cm H_2O, PEEP ≥ 5 cm H_2O) will little influence the incidence and severity of postoperative respiratory complications. Indeed, in this large population group, postoperative atelectasis is the commonest problem and the major cause of hypoxemia and nosocomial pneumonia. Accordingly, preventing atelectasis should be considered as an important objective in perioperative management.[3]

After anesthesia induction in the supine position, functional residual capacity is markedly reduced (approximately 0.7–1.3 l), and atelectasis develops in the dependent part of the lungs as a result of the loss of inspiratory muscle tone, cephalad diaphragm displacement, intrathoracic shift of blood volume, and oxygen resorption.[4] Starting from a lower functional residual capacity, the inspiratory–expiratory cycles are completed on a lesser compliant part of the pressure–volume curve, and the repetitive opening–closing of small airways and unstable alveoli initiate proinflammatory responses. Accordingly, the mechanical breath (V_T) is delivered to a nonhomogenous lung with a continuum ranging from variable degree of alveolar collapse (dependent areas) to a variable degree of overdistension (nondependent areas) that translates into ventilation–perfusion mismatch with impaired oxygenation.

After numerous failed attempts to acquire a Reply from the Editorial authors to this Letter, it is being published without the benefit of their response.—James C. Eisenach, M.D., Editor-in-Chief.

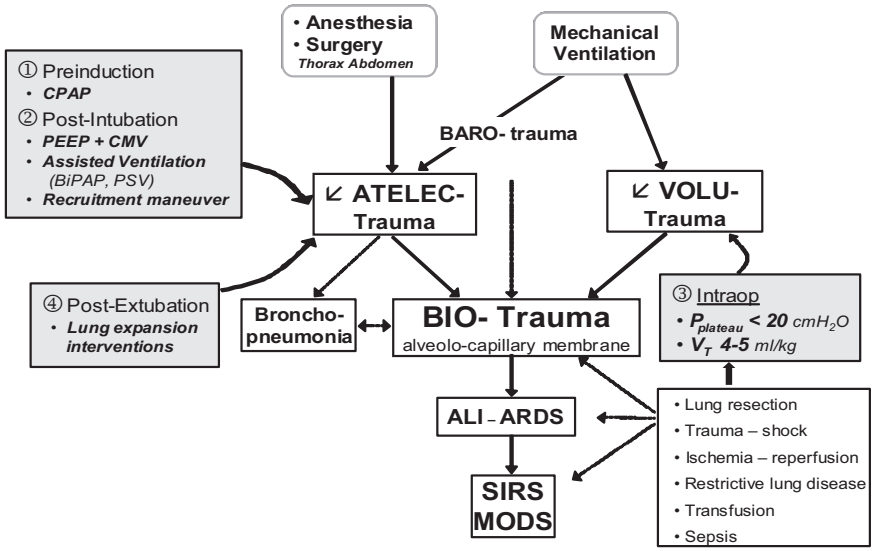

Fig. 1. Perioperative multimodal lung-protective approach. ALI = acute lung injury; ARDS = adult respiratory distress syndrome; BiPAP = bilevel positive airway pressure; CMV = controlled mechanical ventilation; CPAP = continuous positive airway pressure; Intraop = intraoperative; MODS = multiple organ dysfunction syndrome; PEEP = positive end-expiratory pressure; PSV = pressure-support ventilation; SIRS = systemic inflammatory response syndrome; V_T = tidal volume.

Besides limiting alveolar trauma with low V_T, attenuating the loss of functional residual capacity and preventing the formation of atelectasis should be attempted by a stepwise approach (fig. 1): (1) application of continuous positive airway pressure and PEEP during the induction of anesthesia[5,6]; (2) titration of low to moderate PEEP levels according to physiologic indices (lower inflection point of the pressure–volume curve, oxygenation indices, hemodynamics) and/or lung imaging techniques (*e.g.*, electrical thoracic impedance)[7]; (3) intraoperative lung recruitment maneuvers (manual inflation up to the vital capacity, "ramp" PEEP elevation up to 20 cm H_2O)[8]; (4) use of inspiratory oxygen concentration less than 80%; and (5) postoperative lung expansion strategies, including postural changes, early mobilization, and deep breathing exercises, as well as noninvasive ventilatory support.

Whenever possible, partial ventilatory modes (assist-controlled, pressure-support, bilevel positive airway pressure) through facial or laryngeal masks should be considered to avoid tracheal (re)intubation, to reduce the duration of mechanical ventilation, and to promote active displacement of the dependent part of the diaphragm. Intraoperatively, bilevel positive airway pressure ventilation has been shown to improve oxygenation indices by decreasing ventilation–perfusion mismatch.[9] Likewise, reversal of atelectasis and hypoxemia after major thoracic and abdominal surgery has been successfully achieved with noninvasive ventilatory techniques that resulted in a reduced need for reintubation and a lower incidence of pneumonia and sepsis.[10]

To date, further randomized controlled trials are needed to question whether a multimodal lung approach effectively prevents the formation of lung atelectasis and reduces the incidence of other pulmonary complications (pneumonia, respiratory failure, hypoxemia necessitating oxygen therapy) after various types of surgical procedures.

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References

1. Schultz MJ, Haitsma JJ, Slutsky AS, Gajic O: What tidal volumes should be used in patients without acute lung injury? Anesthesiology 2007; 106:1226–31
2. Putensen C, Wrigge H: Tidal volumes in patients with normal lungs: One for all or the less, the better? Anesthesiology 2007; 106:1085–7
3. Turrentine FE, Wang H, Simpson VB, Jones RS: Surgical risk factors, morbidity, and mortality in elderly patients. J Am Coll Surg 2006; 203:865–77
4. Hedenstierna G, Edmark L: The effects of anesthesia and muscle paralysis on the respiratory system. Intensive Care Med 2005; 31:1327–35
5. Rusca M, Proietti S, Schnyder P, Frascarolo P, Hedenstierna G, Spahn DR, Magnusson L: Prevention of atelectasis formation during induction of general anesthesia. Anesth Analg 2003; 97:1835–9
6. von Ungern-Sternberg BS, Regli A, Schibler A, Hammer J, Frei FJ, Erb TO: The impact of positive end-expiratory pressure on functional residual capacity and ventilation homogeneity impairment in anesthetized children exposed to high levels of inspired oxygen. Anesth Analg 2007; 104:1364–8
7. Erlandsson K, Odenstedt H, Lundin S, Stenqvist O: Positive end-expiratory pressure optimization using electric impedance tomography in morbidly obese patients during laparoscopic gastric bypass surgery. Acta Anaesthesiol Scand 2006; 50:833–9
8. Whalen FX, Gajic O, Thompson GB, Kendrick ML, Que FL, Williams BA, Joyner MJ, Hubmayr RD, Warner DO, Sprung J: The effects of the alveolar recruitment maneuver and positive end-expiratory pressure on arterial oxygenation during laparoscopic bariatric surgery. Anesth Analg 2006; 102:298–305
9. Yu G, Yang K, Baker AB, Young I: The effect of bi-level positive airway pressure mechanical ventilation on gas exchange during general anaesthesia. Br J Anaesth 2006; 96:522–32
10. Squadrone V, Coha M, Cerutti E, Schellino MM, Biolino P, Occella P, Belloni G, Vilianis G, Fiore G, Cavallo F, Ranieri VM, Piedmont Intensive Care Units Network (PICUN): Continuous positive airway pressure for treatment of postoperative hypoxemia: A randomized controlled trial. JAMA 2005; 293:589–95

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Anesthesiology 2008; 108:336–7

In Reply:—We thank Dr. Licker *et al.* for their comments on our article about optimal ventilator strategies in patients without acute lung injury.[1] Indeed, overstretching lungs with conventional and abnormally high tidal volumes during surgery has been shown to trigger procoagulant and proinflammatory alveolar responses in patients with healthy lungs, while not being particularly useful to prevent intraoperative atelectasis.[2,3] When we consider the concept of "multiple hits" to explain the pathophysiologic mechanisms of acute lung injury, a protective ventilatory strategy (using "normally sized" tidal volume to prevent lung stretch) is certainly indicated in the management of surgical patients with lungs at risk for lung injury (*e.g.*, with systemic inflammatory response syndrome, major trauma, major surgery). In this context, we would like to stress that the terminology chosen for a strategy aiming at prevention of overstretching the lungs (conventional *vs.* low tidal volumes) is wrong and maybe even misleading. Instead of "lower" tidal volumes, we should use the term "normal" or "normally sized" tidal volumes. It is like traffic speeding: traffic speeding during rush hours is very dangerous—but traffic

speeding is always dangerous, even when there are not so many other cars on the road; therefore, regulations mandate that we never drive faster than the speed limit. The size of a normal tidal volume is approximately 6 ml/kg for all mammals[4]—we should always consider use of normally sized tidal volumes rather than (very) high tidal volumes.

We agree that ventilation with normal tidal volumes as proposed in our review may not prevent the development of postoperative atelectasis. Although limited evidence supports the use of higher positive end-expiratory pressure, intraoperative recruitment maneuvers, lower oxygen fraction, and postoperative noninvasive ventilation,[5] a multimodal lung-protective approach has not been tested.

Although postoperative pulmonary complications are common and associated with significant morbidity, few studies investigated the influence of intraoperative ventilator and nonventilator management (*e.g.*, fluid balance, transfusions). Indeed, randomized controlled trials are needed to answer whether a multimodal lung-protective approach effectively prevents the formation of atelectasis and reduces the incidence of acute lung injury and other pulmonary complications after various types of surgical procedures.

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References

1. Schultz MJ, Haitsma JJ, Slutsky AS, Gajic O: What tidal volumes should be used in patients without acute lung injury? ANESTHESIOLOGY 2007; 106:1226–31
2. Choi G, Wolthuis EK, Bresser P, Levi M, van der Poll T, Dzoljic M, Vroom MB, Schultz MJ: Mechanical ventilation with lower tidal volumes and positive end-expiratory pressure prevents alveolar coagulation in patients without lung injury. ANESTHESIOLOGY 2006; 105:689–95
3. Wolthuis EK, Choi G, Dessing MC, Bresser P, Lutter R, Dzoljic M, van der Poll T, Vroom MB, Hollmann M, Schultz MJ: Mechanical ventilation with lower tidal volumes and positive end-expiratory pressure prevents pulmonary inflammation in patients without preexistent lung injury. ANESTHESIOLOGY 2008; 108:46–54
4. Tenney SM, Remmers JE: Comparative quantitative morphology of the mammalian lung: Diffusing area. Nature 1963; 197:54–6
5. Whalen FX, Gajic O, Thompson GB, Kendrick ML, Que FL, Williams BA, Joyner MJ, Hubmayr RD, Warner DO, Sprung J: The effects of the alveolar recruitment maneuver and positive end-expiratory pressure on arterial oxygenation during laparoscopic bariatric surgery. Anesth Analg 2006; 102:298–305

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Anesthesiology 2008; 108:337

Endotracheal Tube with End-tidal Carbon Dioxide Port

To the Editor:—I read with interest the brief report by Dr. Al-Nabhani *et al.*[1] on problems of monitoring end-tidal carbon dioxide in extremely low-birth-weight infants during perioperative period. For the monitoring of end-tidal carbon dioxide in neonates, I agree that it is necessary to sample alveolar gases to avoid the dilution of carbon dioxide by dead space created by ventilating devices such as the endotracheal tube adaptor, the Y-piece of the breathing circuit, and even the T-piece for carbon dioxide sampling, and it is necessary to insert a catheter into the endotracheal tube for sampling of alveolar gases.

For sampling of alveolar gases without using an endotracheal catheter, an endotracheal tube with end-tidal carbon dioxide monitoring port (Mallinckrodt Inc., St. Louis, MO) is available. As shown in figure 1, the lumen for end-tidal carbon dioxide sampling extends to near the distal end of endotracheal tube. The outside diameter of the 3.0-mm uncuffed tube with monitoring port is 4.5 mm, compared with 4.3 mm for a standard uncuffed tube. Although the endotracheal tube with monitoring port is slightly larger in size by 0.2 mm, the difference is negligible. I have never had any problems with endotracheal intubation. With use of this tube, one can avoid the insertion of the catheter into the endotracheal tube, and hence avoid related complications.

Charles Her, M.D., F.C.C.P., New York Medical College, Valhalla, New York. charles6133@msn.com

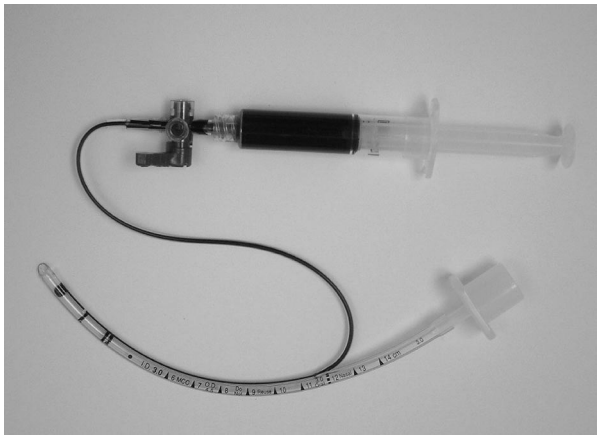

Fig. 1. A 3.0-mm uncuffed endotracheal tube with end-tidal carbon dioxide monitoring port. Methylene blue dye was injected into the end-tidal carbon dioxide monitoring port to visualize the separate lumen. The dye entered the main lumen of the endotracheal tube at the near distal end of tube.

Reference

1. Al-Nabhani D, Shivananda S, Holtby H, McNamara PJ: Hazardous course of an end-tidal carbon dioxide catheter in a premature infant. ANESTHESIOLOGY 2007; 107:180–1

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Anesthesiology 2008; 108:337–8

In Reply:—We are delighted that our report has stimulated some interesting discussion on the challenges of end-tidal carbon dioxide monitoring.[1] Dr. Her describes his experience with a new type of endotracheal tube, which has a built-in end-tidal carbon dioxide monitoring port (Mallinckrodt Inc., St. Louis, MO). We agree that the complication seen in our patient could have been avoided with this form of tube because dislodgement and distal migration are less likely. There are some other advantages that should be noted. Because the monitoring line does not occupy the inner lumen, airway resistance is not increased. This is particularly relevant for very low-birth-weight infants, where 2.0- to 2.5-mm uncuffed endotracheal tubes are commonly used and airway resistance is most likely to be affected. This type of tube can be easily used with an appropriate end-tidal measuring system, provided the sample volume aspirated does not compromise the delivered tidal volume.

There are some limitations that need to be pointed out. The additional tubing may become entangled with other tubes (*e.g.*, nasogastric

tube) or may be pulled on by an active patient, both potentiating an accidental extubation. If the tube is inadvertently cut (*e.g.*, during tube suturing or taping) or the stopcock is left open to the atmosphere, a leak may occur, leading to ventilator autocycling and suboptimal tidal volume delivery. If used chronically for long-standing ventilated patients, the sampling line may become occluded by mucous or water secondary to circuit humidification. Finally, the current cost of the device may prohibit widespread use.

Although continuous end-tidal carbon dioxide is a close measure of arterial carbon dioxide partial pressure in patients with normal ventilation and perfusion, it does not guarantee appropriate tube position.[2] It is most useful for trending or screening patients for abnormal carbon dioxide values. This type of endotracheal tube may be useful for patients requiring short-term procedural intubation in the operating room or during high-risk neonatal transport, where the risk of endotracheal tube migration/dislodgement is high. The reliability of this device needs careful prospective evaluation.

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References

1. Al-Nabhani D, Shivananda S, Holtby H, McNamara PJ: Hazardous course of an end-tidal carbon dioxide catheter in a premature infant. ANESTHESIOLOGY 2007; 107:180–1
2. Noble JJ: Carbon-dioxide monitors: Exhaled gas (capnographs, capnometry, end-tidal CO_2 monitors). Pediatr Emerg Care 1993; 9:244–6

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Anesthesiology 2008; 108:338

Limitations of Genetic Findings That Are Not in Hardy-Weinberg Equilibrium

To the Editor:—Zaugg *et al.*[1] report an association between the Arg389Gly (rs1801253) single nucleotide polymorphism of the β_1-adrenergic receptor and adverse cardiac outcomes occurring within 1 yr of spinal anesthesia in patients with clinically important coronary artery disease. Although quite interesting, this report may be flawed by an important statistical methodology error.

The reported genotypes of rs1801253 are not in Hardy-Weinberg equilibrium ($P = 4.2 \times 10^{-7}$) and have a lower than expected number of heterozygotes. Publicly available genotyping demonstrates that the Arg389Gly genetic variant is generally found to be in Hardy-Weinberg equilibrium.* The most frequent cause of reduced heterozygote expression is genotyping error caused by low amplification of one of the two alleles in the genotyping process. Examination of genotyping intensity plots will frequently, but not always, identify such errors. With such a markedly abnormal result, the authors would be advised to regenotype this single nucleotide polymorphism using another genotyping platform, preferably by an independent laboratory, to confirm their findings.

The reader is referred to an informative description of Hardy-Weinberg equilibrium in the same issue of ANESTHESIOLOGY for an explanation of this important quality control measure in genotyping studies.[2] Guidance for quality control and reporting the results of genotyping studies have recently been provided.[3] Specifically, measures to assess the quality of genotype data should include (1) excluding single nucleotide polymorphisms with low genotyping frequencies, (2) excluding single nucleotide polymorphisms not in Hardy-Weinberg equilibrium, (3) performing genotyping on known study sample duplicates or publicly available samples to confirm accuracy of the genotyping methods, and (4) other methodologic and statistical techniques to ensure data quality. Accordingly, the association reported by Zaugg *et al.* should be regarded with considerable caution until confirmation in other cohorts.

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References

1. Zaugg M, Bestmann L, Wacker J, Lucchinetti E, Boltres A, Schulz C, Hersberger M, Kälin G, Furrer L, Hofer C, Blumenthal S, Müller A, Zollinger A, Spahn DR, Borgeat A: Adrenergic receptor genotype but not perioperative Bisoprolol therapy may determine cardiovascular outcome in at-risk patients undergoing surgery with spinal block: The Swiss Beta Blocker in Spinal Anesthesia (BBSA) study: A double-blinded, placebo-controlled, multicenter trial with 1-year follow-up. ANESTHESIOLOGY 2007; 107:33–44
2. Lötsch J: Basic genetic statistics are necessary in studies of functional associations. ANESTHESIOLOGY 2007; 107:168–9
3. NCI-NH GRI Working Group on Replication in Association Studies, Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Hirschhorn JN, Abecasis G, Altshuler D, Bailey-Wilson JE, Brooks LD, Cardon LR, Daly M, Donnelly P, Fraumeni JF, Freimer NB, Gerhard DS, Gunter C, Guttmacher AE, Guyer MS, Harris EL, Hoh J, Hoover R, Kong CA, Merikangas KR, Morton CC, Palmer LJ, Phimister EG, Rice JP, Roberts J, Rotimi C, Tucker MA, Vogan KJ, Wacholder S, Wijsman EM, Winn DM, Collins FS: Replicating genotype-phenotype associations. Nature 2007; 447:655–60

* http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1801253. Accessed July 19, 2007.

(Accepted for publication October 24, 2007.)

Anesthesiology 2008; 108:338–9

In Reply:—We thank Drs. Body and Schwinn for their critical remarks on our study.[1] To clarify, our double-blinded, placebo-controlled trial was primarily designed to test the hypothesis that the perioperative administration of bisoprolol would reduce the incidence of cardiovascular complications in patients with or at risk for coronary artery disease undergoing surgery with spinal nerve block. However, because single nucleotide polymorphisms of the β-adrenergic receptor genes may act as disease modifiers,[2] biologically important nonsynonymous coding variants of the β-adrenergic receptor were determined in our study population. This analysis showed that carriers of at least one Gly allele of the β_1-adrenergic receptor polymorphism Arg389Gly experienced a higher number of adverse events than Arg homozygotes.[1] Drs. Body and Schwinn raised concerns with respect to genotyping errors based on the observed deviation from Hardy-Weinberg equilibrium for this particular polymorphism in our trial. Genotyping error is indeed one of the many possible sources of Hardy-Weinberg disequilibrium. To exclude this possibility in our trial, we have carefully regenotyped all patients for the Arg389Gly polymorphism using internal controls for wild-type, heterozygous, and mutant

genotypes for each amplification process. As reported in our publication, from the 189 patients, who consented to genotyping, 186 could be unequivocally identified and confirmed by regenotyping by three independent individuals. In three patients (notably without a primary outcome), genotyping was not possible, and these patients were excluded from genotype-related outcome analysis. Our genotyping platform was further meticulously validated by bidirectional sequencing of DNA samples for the Arg389Gly polymorphism from 12 randomly selected patients of this particular study and from many other patients not related to this study. Bidirectional sequencing is regarded as the standard of genotyping and as by far more reliable than any other genotyping platform.

Although testing for Hardy-Weinberg equilibrium is used as some quality-control measure, particularly in case–control gene association studies, it cannot be used to detect genotyping error.[3,4] Genotyping errors are generally small and do not generate sufficient deviations from Hardy-Weinberg equilibrium to be detected. In the case of a reduced number of observed heterozygous patients, as may occur in the presence of poor amplification of one of the alleles, large samples sizes are necessary to detect deviations from Hardy-Weinberg equilibrium. For example, with the Gly389 allele of the Arg389Gly polymorphism with a reported allele frequency of 0.27 and an error rate of 0.05, more than 8,000 patients would be necessary to detect deviation from Hardy-Weinberg with a power of 0.80 at an α level of 0.05. Increasing the error rate to 0.15 reduces the sample size to the still considerably high patient number of 944. Therefore, testing for Hardy-Weinberg equilibrium is an unreliable tool to identify genotyping errors. Conversely, the presence of Hardy-Weinberg equilibrium does not rule out that genotyping errors might have occurred. Hence, it seems unlikely that genotyping error is the source of the Hardy-Weinberg disequilibrium observed in our study. Because approximately 10% of all genotype–phenotype association studies show deviation from Hardy-Weinberg equilibrium, the results of our trial cannot be considered "abnormal."[5] Rather, as outlined in the discussion of our findings,[1] a selection bias (population stratification) may have occurred because of inclusion and exclusion criteria of this randomized trial. A mortality bias (different survival of marker genes) due to varying genetic and environmental background (*e.g.*, response to cardiovascular medication) in this elderly study population at the end of life expectancy may have also caused this disequilibrium. Of note, Hardy-Weinberg disequilibrium that is caused by most interesting biologic phenomena typically results in excess homozygosity, as observed in our study.[1] However, we agree with Drs. Body and Schwinn that violation of Hardy-Weinberg equilibrium in our study population implies a selected rather than a random sample, invalidating direct comparisons with other populations. Therefore, we share their view that our results should be regarded with caution. Our findings should be confirmed in future prospective larger-scale clinical trials, specifically designed and adequately powered to detect genotype-specific differences in cardiovascular outcome in patients with or at risk of coronary artery disease.

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References

1. Zaugg M, Bestmann L, Wacker J, Lucchinetti E, Boltres A, Schulz C, Hersberger M, Kalin G, Furrer L, Hofer C, Blumenthal S, Muller A, Zollinger A, Spahn DR, Borgeat A: Adrenergic receptor genotype but not perioperative bisoprolol therapy may determine cardiovascular outcome in at-risk patients undergoing surgery with spinal block: The Swiss Beta Blocker in Spinal Anesthesia (BBSA) study: A double-blinded, placebo-controlled, multicenter trial with 1-year follow-up. Anesthesiology 2007; 107:33–44

2. Zaugg M, Schaub MC: Genetic modulation of adrenergic activity in the heart and vasculature: Implications for perioperative medicine. Anesthesiology 2005; 102:429–46

3. Zou GY, Donner A: The merits of testing Hardy-Weinberg equilibrium in the analysis of unmatched case-control data: A cautionary note. Ann Hum Genet 2006; 70:923–33

4. Leal SM: Detection of genotyping errors and pseudo-SNPs *via* deviations from Hardy-Weinberg equilibrium. Genet Epidemiol 2005; 29:204–14

5. Trikalinos TA, Salanti G, Khoury MJ, Ioannidis JP: Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. Am J Epidemiol 2006; 163:300–9

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Anesthesiology 2008; 108:339

Newborns and Anesthetic Neurotoxicity

To the Editor:—We read with interest Dr. Anand's Editorial View, "Anesthetic Neurotoxicity in Newborns: Should We Change Clinical Practice?" but we are concerned that readers may misinterpret his indication regarding postnatal day 35 (P35) rhesus monkeys: "anesthetic neurotoxicity primarily results from apoptosis in rodents, . . . whereas infant monkeys at P5 (but not at P35) exhibit both excitotoxicity and apoptosis."[1] Although the report referenced by Dr. Anand in regard to P35 monkeys did not find a neurotoxic effect, it used one control and one experimental sample of n = 3.[2] For each indicator of neurotoxicity, the SDs bracketing the observed results were far greater than the observed difference between the control group and the anesthetized group, such that a confidence interval around each observed difference includes levels of neurotoxicity that cannot be dismissed. Accordingly, the neurotoxicity of anesthetics has not been established beyond age P5 in a primate model. Absence of evidence is (still) not evidence of absence.[3]

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References

1. Anand KJ: Anesthetic neurotoxicity in newborns: Should we change clinical practice? Anesthesiology 2007; 107:2–4

2. Slikker W, Zou X, Hotchkiss CE, Divine RL, Sadovova N, Twaddle NC, Doerge DR, Scallet AC, Patterson TA, Hanig JP, Paule MG, Wang C: Ketamine-induced neuronal cell death in the perinatal rhesus monkey. Toxicol Sci 2007; 98:145–58

3. Hartung J, Cottrell JE, Giffin JP: Absence of evidence is not evidence of absence. Anesthesiology 1983; 58:298–300

(Accepted for publication October 5, 2007.)

The above letter was sent to the authors of the referenced report. The authors did not feel that a response was required.—James C. Eisenach, M.D., Editor-in-Chief.