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Safety of Investigative Bronchoscopy in the Severe Asthma Research Program

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SARP Manual of Operations for Investigative Bronchoscopy. The Bronchoscopy Section (9) of the SARP Manual of Operations is provided in it's entirety in a separate PDF file. Below is a brief table of bookmarks to assist your review of this document. The shaded rows refer to guidelines for pediatric subjects; only one pediatric subject is included in the current analysis.

TABLE E1. THE SARP BRONCHOSCOPY MANUAL OF PROCEDURES

Section	Pages	Brief Description	Comments in reference to current Manuscript
9.1	103	Adult Standards	
9.1.2	103	Subject Selection and Characterization	5 COPD subjects underwent bronchoscopy, but are not included in this analysis.
9.1.3	105	Inclusion/Exclusion Criteria	
	107	Definition of Very Severe Asthma	
	108-109	Phase-In Criteria	
9.1.4	109-110	General Exclusion Criteria Criteria to delay bronchoscopy	
9.1.5	111	Medication dose limits	
9.1.6	111-112	Topical Anesthesia dose limits	
9.1.7	113	Procedural Monitoring	
9.1.8 – 9.1.12	113-115	Bronchoscopy procedures	Procedures for sharing of bronchoalveolar lavage fluid and endobronchial brushings and biopsies between sites.
9.1.13	116	Post-procedure monitoring	
9.1.14	117	Hospitalization Indicators	
9.2	118-121	Safety Algorithm	
9.3	121-123	Pediatric Standards: 6-11 Years of Age	Investigative bronchoscopy was not performed in this age group. The MoP details sample collection from diagnostic bronchoscopies performed for clinical purposes.
9.4	123-133	Pediatric Standards: 12-17 Years of Age	Only one adolescent had been studied at the time of this analysis. To date, three adolescents have undergone investigative bronchoscopy at one center (Washington Univ.)
9.5	133-137	Stopping Rules and DSMB Oversight	
9.6	137-142	Transbronchial Biopsies	One investigator (Dr. Wenzel) performed this procedure.
9.7	142	Approval to Proceed with Bronchoscopy	

TABLE E2: DISTRIBUTION OF BRONCHOSCOPY SUBJECTS AT CENTERS

Center*	Normal	Not Severe Asthma	Severe Asthma	Very Severe Asthma	Center Total
Brigham & Women's Hospital	0	17	12	3	32
Cleveland Clinic	19	17	9	1	46
Imperial College, UK	0	20	20	0	40
National Jewish Hospital	21	17	11	11	60
University of Pittsburgh	27	15	6	21	69
University of Virginia	3	5	2	0	10
Wake Forest University	0	35	9	0	44
University of Wisconsin	17	57	16	2	92
Washington University	10	13	17	3	43
Subject Group Total	97	196	102	41	436

*Emory University is a pediatric clinical site that does not perform investigative bronchoscopy.

Protocol Deviations and Changes to the MoP. Early in SARP an interim data analysis revealed that the total lidocaine dose limit (600 mg or 9 mg/kg whichever is less) was inadvertently exceeded in twenty-two subjects (12 normal, 6 not severe and 4 severe asthma subjects). These subjects received total doses of lidocaine that ranged from 620 to 940 mg, largely due to higher doses of atomized lidocaine applied to the upper airway (which was included in the total reported dose). There were no adverse effects related to these higher doses of lidocaine. Subsequent review of topical anesthesia protocols at all sites (which varied with regard to the use of atomized, gargled or nebulized lidocaine to anesthesize the airways), led to an amendment to the SARP MoP to standardize the reporting of lidocaine doses. Following this change to the protocol, all lidocaine doses are reported as the arithmetic total of lidocaine administered by any route, regardless of whether application is primarily above or below the vocal cords. Lidocaine doses in the current manuscript reflect the total dose of lidocaine given to the subjects during preparation and performance of the procedure.

Section 9

Bronchoscopy Standards

9.1 Adult Standards

9.1.1 Purpose

It is the main goal of the Bronchoscopy Subcommittee to maximize the safety of subjects participating in the SARP. The secondary goal of the Subcommittee is to facilitate the acquisition of airway samples (BAL fluid, BAL cells, bronchial brushings, and bronchial biopsies) that can be shared with other SARP investigators as *shareable samples*. Although it is desirable to procure *shareable samples* for study by other SARP investigators, this objective should not take precedence over the needs of individual investigators to accomplish the scientific goals outlined in their individual grant. The following aspects of invasive bronchoscopic procedures should be adhered to carefully by each investigator when they tailor specific techniques and protocols.

This document presents 2 protocols: severe asthma and very severe asthma. These protocols are presented together to expedite their review and to highlight their differences. Because research in children differs from research in adults, this document omits the *Pediatric Standards*.

9.1.2 Subject Selection and Categorization

Subjects selected for study can fall into one of three disease categories, one of which (asthma) has three subdivisions. As a result, there will be 5 categories of SARP subjects: healthy controls (HC), mild-to-moderate asthma (MMA), severe asthma (SA), very severe asthma (VSA), and chronic obstructive pulmonary disease (COPD). Definitions of HC, MMA, and COPD can vary from site to site. The distinction between MMA and SA can vary also from site to site; in general, however, subjects with MMA exhibit acceptable clinical asthma

control with common asthma controller medications. This section focuses on *1)* the distinction between SA and VSA because the VSA group requires additional safety procedures, *2)* exclusion criteria that differentiate VSA from exclusion due to asthma severity, and *3)* considerations for bronchoscopy in the 5 subject groups. Generally, subjects in HC, MMA, and SA categories can be studied using generally accepted safeguards. Subjects with VSA require additional safeguards and vigilance. Figure 9.1 depicts the relationships among the study groups.

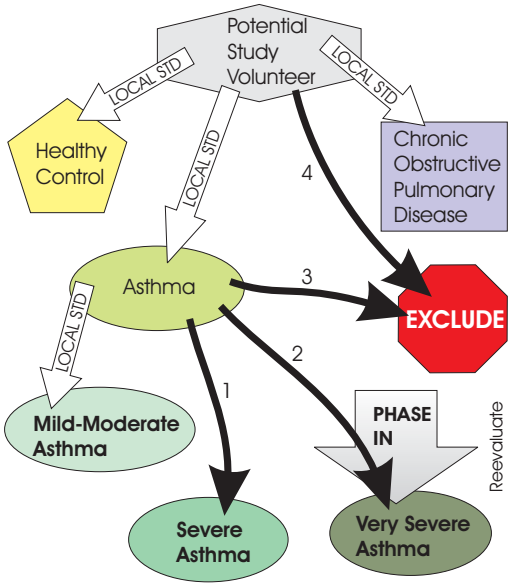

Figure 9.1: There will be 5 groups of SARP volunteers: healthy, chronic obstructive pulmonary disease, mild-to-moderate, severe, and very severe asthma. Because the research questions and control populations will vary, the definitions of healthy, chronic obstructive pulmonary disease, and mild-to-moderate asthma at each center will vary. In contrast, severe asthma has been defined: 1 · see INCLUSION CRITERIA FOR ALL ASTHMATIC SUBJECTS (p 106). Very severe asthma has also been defined: 2 · see CRITERIA FOR DISTINGUISHING SA FROM VSA (p 107). For subject safety, there are clear exclusion criteria (Section 9.1.4, p 109). These criteria may exclude subjects with more severe asthma depending on their phase-in criteria: 3 · see PHASE IN CRITERIA FOR BRONCHOSCOPY IN VERY SEVERE ASTHMA (p 108). A clinically important comorbidity is an exclusion: 4 · see EXCLUSION FROM BRONCHOSCOPY FOR AN INDEFINITE PERIOD (p 110). The inclusion of subjects with progressively more severe disease into the very severe asthma group is depicted by the Phase-In arrow; monitoring by the DSMB will occur during the Phase-In period. Local STD, local standards.

An algorithm for caring for subjects who participate as bronchoscopy volunteers has been developed and incorporated into a flow sheet (Section 9.2).

Subjects must meet all inclusion criteria (Section 9.1.3) and must fail to meet all exclusion criteria (Section 9.1.4). Summary tables of inclusion and exclusion criteria are reproduced here.

Table 9.1: General Inclusion Criteria for Asthma Subjects

- Asthma is present, defined by site-specific criteria, and has been classified as MMA, SA, or VSA
- FEV_1 is within acceptable limits (Table 9.3)
- Informed consent is present
- Bronchoscopist has determined that subject is clinically appropriate for bronchoscopy

n.b. This summary provides overview, and is not exhaustive in detail. See Section 9.1.3.

Table 9.2: General Exclusion Criteria for Asthma Subjects

- FEV_1 is less than 35% predicted before, or less than 40% predicted after, bronchodilator administration. This criterion is the most inclusive limit. Additional restrictions may apply. See Table 9.3 .
- Asthma is clinically unstable (Section 9.1.4)
- Communication channel is not established for follow-up contacts
- Clinically significant, unstable comorbidities are present

n.b. This summary provides overview, and is not exhaustive in detail. See Section 9.1.4.

Considerations for all subjects

- Ability to cooperate with procedures
- Ability to give consent
- Clotting studies, CBC, and platelet counts may be done as a bronchoscopy screen at the discretion of investigators, but are not required.
- Coexisting lung disease

Considerations for asthmatic subjects

- Clinical stability, consistent with the specific inclusion and exclusion criteria outlined below. A subject judged to be clinically unstable by the investigator or bronchoscopist should generally be excluded from bronchoscopy until acceptable clinical stability, as defined in the inclusion and exclusion criteria (Sections 9.1.3 and 9.1.4), is achieved.
- For participants with severe asthma, or very severe asthma, a competent companion or caretaker should be available at home. Identification of such a person should precede initiation of sedation and bronchoscopy.

- For participants with very severe asthma, additional screening, procedural, monitoring, and follow-up considerations are recommended.

9.1.3 Inclusion Criteria for Asthma (MMA, SA, VSA), COPD, and Healthy Controls

Healthy control (HC) volunteers must meet site-specific requirements for inclusion. Generally, these requirements will include no history of allergies or asthma, normal spirometry, and a trivial or absent smoking history.

Volunteers with chronic obstructive pulmonary disease (COPD) must meet site-specific requirements for inclusion. Generally, these requirements will include a significant tobacco smoking history, and evidence of fixed airflow obstruction. Allergies may or may not be present, but a formal history of asthma is absent.

Volunteers with asthma will meet site-specific criteria, which generally include the demonstration of airflow obstruction which may be reversible with β-agonists. A physician diagnosis of asthma is often present prior to subject enrollment in the SARP. Allergies may or may not be present.

Inclusion Criteria for All Asthmatic Subjects

Subjects must meet each of the following criteria in order to qualify to participate in bronchoscopic study at any level of asthma severity. These criteria, with those of Section 9.1.4 , form the border between VSA and Exclude categories in Figure 9.1 .

- PFT: prebronchodilator FEV_1 in *Spirometry 1*, obtained prior to bronchodilator administration, must always be $\geq 35\%$ predicted. As detailed in Table 9.3 , we will begin with a floor FEV_1 criterion of $\geq 45\%$, prior to bronchodilator, and phase in more severe subjects based on objective evidence of safety and experience, and with monitoring by the Bronchoscopy Subcommittee and the DSMB.
- PFT: postbronchodilator FEV_1 in *Spirometry 2*, obtained after administration of 2.5 mg albuterol by nebulizer, in all cases must be $\geq 40\%$ predicted. As detailed in Table 9.3 , we will begin with a floor FEV_1 criterion of $\geq 60\%$, following bronchodilator, and phase in more severe subjects based on objective evidence of safety and experience, and with monitoring by the Bronchoscopy Subcommittee and the DSMB.
- Age: for adult protocols, age of $\geq$ 18 years and $\leq$ 60 years. Due to the increasing incidence of comorbidities, and the potential stress of the invasive procedure of bronchoscopy, the age of 60 is selected as conservative and biased in the direction of subject safety. Exceptions to the 60-year limit

may be made on a case-by-case basis, for individuals who demonstrably are free of important comorbidities (see Section 9.4.3, p 110). For those individuals older than 60, an ECG and CXR will have been obtained within the past 6 months. Exceptions for subjects older than > 60 years of age are expected to be uncommon.

- Gender: no selection criteria
- Minority status: no selection criteria
- No hospitalization for asthma within 6 weeks, and no intubation for asthma within 6 months.
- Subject must be judged otherwise to be clinically appropriate for bronchoscopy by bronchoscopist. No specific PFT or other criteria, other than above, are used to document acceptability and appropriateness. Safety of the subject is the overriding concern in making this determination. Details of the clinical course must be detailed in the record.

General Approach to Subject Safety: Rationale for Distinguishing Severe Asthma from Very Severe Asthma

Asthma is temporally variable. Thus, a specific subject may at one time exhibit selection parameters consistent with SA, but at time of bronchoscopy be consistent with VSA. Subjects with VSA may demonstrate considerably greater physiologic brittleness or volatility, and may decompensate acutely with or without warning signs. Thus, the determination of the level of asthma severity, and the consequential procedural and monitoring safeguards which should be employed, must be reassessed on the day of bronchoscopy. Criteria to make this distinction are listed in Section 9.1.3 .

Criteria for Distinguishing SA from VSA: VSA/SA Border Criteria

For subjects who otherwise meet criteria for inclusion in the SARP for bronchoscopy, the presence of any of the following characteristics places the subject into the category of Very Severe Asthma, rather than Severe Asthma:

- $FEV_1 < 60\%$ predicted following administration of 2.5 mg albuterol via nebulizer.
- Use of short-acting β-agonists in excess of 8 puffs per day within 48 hours of bronchoscopy
- In the presence of regular long-acting β-agonists, the use of short-acting β-agonists in excess of 4 puff per day within 48 hours of bronchoscopy
- Hospitalization for asthma within 6 months
- Endotracheal intubation for asthma within 1 year

- More than 6 exacerbations in the past 180 days (about one per month)
- Changing dose of oral corticosteroids (up or down) in the past 14 days
- Regular oral corticosteroid dose of 20 mg/day or more
- Asthma exacerbation or other clinical instability in the judgment of the investigator or bronchoscopist
- D_{LCO} may be performed at the discretion of the investigator to clarify the diagnosis if necessary. In this setting, a D_{LCO} of $< 70\%$ predicted will be exclusionary for any asthma group. However, determination of D_{LCO} is not mandatory.

Phase In Criteria for Bronchoscopy in Very Severe Asthma: Exclude/VSA Border Criteria

The SARP investigators place participant safety in first place on our program agenda. Consequently, we will phase in the study of the most severe subjects using conservative criteria initially, with an increasingly inclusive protocol, based on the individual site experience of the SARP investigators, and the oversight of the DSMB.

During the first phase of subject recruitment, all subjects must demonstrate an FEV_1 of at least 45% predicted prior to administration of albuterol, as above, and at least 55% predicted after albuterol. Note that subjects with FEV_1 at least 55% predicted following bronchodilator are generally designated as severe asthma and not very severe asthma. Thus, this phase-in approach effectively eliminates recruitment of VSA subjects until a center has experience in SA. Accrual and experience of asthma subjects will be recorded on a site-by-site (10 SARP sites, 8 SARP centers) basis. The accrual of 7 qualifying subjects at a specific site will constitute *Phase 1* . At the completion of *Phase 1* , the center will ask the Bronchoscopy Subcommittee to review the safety experience in their initial 7 subjects. If approved by the Bronchoscopy Subcommittee, DSMB approval will be sought. On DSMB approval, *Phase 2* will begin. During *Phase 2* , all subjects with VSA must demonstrate an FEV_1 of at least 40% predicted prior to administration of albuterol, as above, and at least 50% predicted after albuterol. These subjects will have more significant airway obstruction, but will still have clinically important β-agonist induced reversibility of the obstruction. After the accrual of 7 subjects meeting this criterion at a specific center, the safety experience will be submitted to the Bronchoscopy Subcommittee and DSMB for review and approval to move to *Phase 3* for the duration of the SARP projects. In *Phase 3* , all subjects with VSA must demonstrate an FEV_1 of at least 35% predicted prior to administration of albuterol, as above, and at

least 40% predicted after albuterol. Safety data from these subjects collected during *Phase 3* will be submitted to the DSMB for review every 10 subjects enrolled at each center. Table 9.3 summarizes these criteria.

Table 9.3: Absolute FEV_1 Criteria for Inclusion in the Very Severe Asthma Category*

	Phase 1, SA 7 Qualifying subjects	*Phase 2*, VSA 7 Qualifying subjects	*Phase 3*, VSA Until completion
FEV_1 prealbuterol	$\geq$ 45% predicted	$\geq$ 40% predicted	$\geq$ 35% predicted
FEV_1 postalbuterol	$\geq$ 55% predicted	$\geq$ 50% predicted	$\geq$ 40% predicted
Center sample size†	7	7	10‡

∗ To be included in a category, a subject must meet both criteria. Reversibility criteria have no bearing on inclusion.

† To qualify for DSMB review and study of next phase of severity

‡ DSMB safety review after every 10 subjects with very severe asthma

9.1.4 General Exclusion Criteria

- Inability to provide informed consent
- Inability or unwillingness to complete schedule SARP procedures
- Lack of reliable communications channel (hard-wire phone, cell phone, email for follow-up contacts after bronchoscopy)

Exclusion from SARP Enrollment

The following exclusion criterion should exclude asthmatic and normal subjects, but not COPD subjects, from enrollment in SARP: Subjects with current smoking history, or smoking history within one year, are excluded; former smokers with < 5 pack-year total history, who have been abstinent for at least one year may be included at the discretion of the investigator.

Exclusion from Bronchoscopy for 14 Days

The presence of any of the following characteristics that differentiate VSA from temporary exclusion will exclude for 14 days a subject from participating as a bronchoscopy volunteer:

Events occurring within 6 months of bronchoscopy

- Intubation for asthma within the past 6 months
- More than 12 exacerbations within the past 6 months

Events occurring within 6 weeks of bronchoscopy

- Hospitalization for asthma within the past 6 weeks

Events occurring within 2 weeks of bronchoscopy

- Increased oral corticosteroid use in the past 14 days, recognized as a dose which is both numerically at least twice that of baseline, and which is at least 20 mg/day greater than the baseline dose

Events occurring within 48 hours of bronchoscopy

- Pulse oximetry demonstrating oxygen saturation $< 90\%$ on room air
- Use of more than 16 puffs of a short acting β-agonist per day in the past 48 hours
- Significant increase in asthma symptoms in the past 48 hours, recognized as an increased use of short acting β-agonists of more than 8 puffs/day (more than 8 puffs/day over baseline)
- $FEV_1 < 40\%$ predicted after administration of 2.5 mg albuterol via nebulizer; n.b. the minimal operative FEV_1 may be higher than this absolute cutoff. See Table 9.3 for phase-in criteria.
- $FEV_1 < 35\%$ predicted before the administration of bronchodilator; n.b. the minimal operative FEV_1 may be higher than this absolute cutoff. See Table 9.3 for phase-in criteria.

Exclusion from Bronchoscopy for an Indefinite Period

The presence of clinically important comorbidities—these include uncontrolled diabetes, uncontrolled coronary artery disease, acute or chronic renal failure, and uncontrolled hypertension—that would increase the risk of significant adverse events during bronchoscopy represent exclusion criteria for bronchoscopy. If the comorbidity resolves, the subject's eligibility can be reevaluated. The clinical judgment of the investigator or bronchoscopist is required, and in those cases in which judgments must be made, bias towards subject safety is essential.

9.1.5 Medication for Bronchoscopy

As the details of medication are unlikely to affect shared samples, this aspect of the procedure is left to the discretion of the investigator and bronchoscopist.

Conscious sedation, delivered by IV medication, or light anxiolysis delivered by a route other than IV, may be employed at the discretion of the investigator and as approved by the IRB of the investigators. Clinical judgment is critically important for selecting and administering conscious sedation. Dose titration is the rule, rather than the exception, and fixed dosing regimens are neither commonly used clinically, nor biologically justifiable. For sites that use IV conscious sedation, all relevant local policies and the SARP procedures detailed in the *Manual of Procedures* (Section 9.2, p 118) will be observed.

For sites that use light parenteral sedation, we will offer this form of sedation to all subjects. If subjects refuse this sedation, we believe they may do so without compromise.

In subjects with VSA, IV access is recommended, whether or not IV conscious sedation is employed.

Table 9.4 lists commonly used medications and adult dose ranges.

Table 9.4: Medications for Bronchoscopy

Class	Drug	Dose Range
Anxiolytic	Midazolam (IM or IV)	0.5–10 mg
Bronchodilator	Albuterol (nebulized)	2.5–5 mg
Anticholinergic	Atropine (IM or IV)	0.4–0.6 mg
	Glycopyrrolate (IM or IV)	0.2–0.4 mg
Narcotic-Antitussive	Demerol (IM or IV)	50–100 mg
	Fentanyl (IM or IV)	25–250 μg
	Codeine (IM or PO)	30–60 mg
	Alfentanyl (IM or IV)	250–500 μg
	Morphine (IM or IV)	2–8 mg

9.1.6 Topical Anesthesia

As the details of topical anesthesia are unlikely to affect shared samples, this aspect of the procedure is left to the discretion of the investigator and bronchoscopist. The spirit of this guideline is to minimize the lidocaine dose delivered to the volunteer. For purposes of calculation, all lidocaine administered is to be included, whether delivery is via gargle, aerosol, spray, or instillation. For

subject safety, the

total lidocaine limit will be 600 mg.

This limit is based on a recent publication[1] that demonstrates the safety of this dosing regimen and acceptable serum lidocaine levels. If this limit is exceeded, a serum lidocaine level should be obtained and the subject observed. The amount of lidocaine used should be recorded in the procedure record sheet.

9.1.7 Procedural Monitoring

If no IV conscious sedation is employed, minimum monitoring includes O_2 saturation and heart rate.

If IV conscious sedation is employed, minimum monitoring includes O_2 saturation, heart rate, BP, and ECG. If conscious sedation is employed, all local policies relevant to conscious sedation should be observed.

Note: if *any sedation* is administered, follow post-bronchoscopy procedures for the subject's disease classification as described in this *Manual of Procedures*.

Personnel Recommendations

To ensure subject safety, and to facilitate appropriate processing of samples, the following minimum in suite personnel are recommended for all procedures:

- Bronchoscopist
- Bronchoscopy Assistant (Nurse, RT, or trained technician); this person should assist the bronchoscopist in monitoring the subject for signs of physiologic distress
- Sample handler
- In subjects with VSA, an additional trained individual is recommended; the sole task of this person is to monitor vital signs, oximetry, and other physiologic and clinical signs to ensure subject safety.

Procedural Documentation

In all cases, documentation of details of the procedure is required. Use of a site-specific standard form for all procedures is strongly encouraged, in order to capture all relevant clinical issues; the details of the form will be institution specific. An appropriate form is available from the DCC website. The following information should be captured:

[1] Langmack EL, et al. *Chest* 2000; 117: 1055–60.

Prior to Bronchoscopy

Subject status: HC, MMA, SA, VSA, COPD
Mental status
Pulmonary examination
Vital signs
Baseline pulse oximetry
Investigator review of recent H&P and *SARP Prebronch Checklist*

Note: For subjects with VSA, or for subjects for whom the bronchoscopist or investigator determines an *a priori* need for overnight hospitalization and monitoring, *documentation of this decision before the procedure* is essential to avoid the requirement to report such hospitalization as a serious Adverse event.

Procedural Details

Bronchoscopist and assistants
Premedications: dose and route
Topical anesthesia: type, dose
Route (oral or nasal)
Appearance of vocal cords
Sites of procedures (BAL, brushings, biopsies)
BAL: aliquot size (standard at 50ml), number (standard 2), volume return
Bronchial brushings: location
Biopsies: number and location

Procedural Monitoring

O_2 saturation and heart rate
Add BP and ECG if conscious sedation is used

Postprocedure

Document acceptable O_2 saturation on room air
Document FEV_1 within 15% of prebronchoscopy, prealbuterol value
Document 24/7-valid contact info provided to volunteer
Document determination of need for extended 12+ hour monitoring or hospitalization (see Sections 9.1.13 and 9.1.14)

9.1.8 Approach

The selection of transoral or transnasal approach will be left to the discretion and judgment of the bronchoscopist, based on individual subject characteristics and anatomy, choice of bronchoscope, and other local considerations. The approach route must be documented in the procedure record.

9.1.9 Bronchoalveolar Lavage: Performance

Bronchoalveolar lavage will often be the first procedure performed (of BAL, brushings, and biopsies) because the latter two generate trauma which will contaminate BAL fluids, even if the BAL is performed in a segment distinct

from the brushings or biopsies. However, the needs and preferences of the individual investigators should be honored. If biopsies are performed prior to BAL, care must be taken to clear the working channel of the bronchoscope of contaminating blood and other debris.

Shareable samples of BAL should be obtained prior to bronchial brushings or bronchial biopsy, or taken from a different segment, and preferably a different lobe, in order to avoid contamination of the sample with blood.

In order to ensure maximal volume return, fluid to be used for BAL will be warmed (37°C). Instillation of fluid will occur using hand pressure on a syringe, and the fluid will be recovered into the same syringe using hand suction to minimize mucosal trauma and contamination of BAL fluid with serum proteins.

BAL will employ 50 ml aliquots. The standard technique will be instillation and recovery of one aliquot, followed by instillation and recovery of the second aliquot. If the recovered volume is < 15 ml from the first 100 ml of injectate, then the BAL will be stopped. The return from these 2 syringes will be pooled, and the supernatant recovered for analysis. Investigators who choose to use higher volume BAL are free to do so with additional 50 ml aliquots. However, the combined return from the first two, as noted above, will be considered the standard *shareable samples* BAL material.

Standard technique will be to use two 50-ml aliquots, pooled and frozen; additional aliquots may be obtained from the same segment at the discretion of the investigator, based on the needs of the study. Further, additional segments may be used, again at the discretion of the bronchoscopist.

9.1.10 Bronchoalveolar Lavage: Processing

The return from the first 2 syringes will be pooled. No straining of fluid through gauze or wire mesh should be performed, in order to minimize loss of cells. Fluid is centrifuged to recover cell pellet. The excess of the supernatant beyond the needs of the investigator is aliquoted in as many as ten 1-ml samples (*shareable samples*). An additional 1 ml aliquot may be prepared for the Cleveland site. Another additional 1 ml aliquot, mixed with an equal volume of ice cold methanol, may be prepared for the Boston site.

The cell pellet is resuspended, cell counts are performed, and 12 cytocentrifuge slides are prepared. Two are stained with Diff-Quick or other histological stain and are interpreted for cellular differential. From this information, total and differential cell counts are computed. Of the other 10 slides, 5 are fixed in acetone, and 5 are fixed in 0.5% formalin; these latter 10 slides represent *shareable samples*.

9.1.11 Bronchial Brushings

In most circumstances, BAL procedures should be completed prior to brushings, in order not to contaminate the BAL with red blood cells or other brush-related traumatic artifacts.

Locale of brushing is at the discretion of bronchoscopist, but typically is performed in a lower lobe segment, with 10 fore-and-aft strokes performed to dislodge epithelial cells. Cells may be dislodged by agitation in sterile media, and recovered by centrifugation, or by preparing a direct smear onto a sterile slide.

Epithelial cells in excess of those required for the project of the investigator may be used to prepare *shareable samples*. To do so, prepare up to 4 cytocentrifuge slides with $50,000$ to $75,000$ cells per 0.5 ml, or $200,000$ to $300,000$ cells total). Fix 2 in methanol. Fix 2 in 0.5% paraformaldehyde. An additional slide may be prepared by smearing the brush across a glass slide, and fixing with 0.5% paraformaldehyde (Cleveland site).

9.1.12 Bronchial Biopsy

Bronchial biopsy is usually performed after BAL; however, the specific order or procedures may vary site-to-site depending on the specific needs of the project at the site. Locale for biopsy is at the discretion of the bronchoscopist. Endobronchial biopsies should be performed under direct vision, rather than blindly. The DSMB recommends that the location from which biopsies are taken be recorded in the procedure record. Transbronchial lung biopsy is a single-institution technique, and will not be addressed herein.

Forceps should be sharp; disposable units are preferable. Alligator forceps are preferred to cup forceps due to less crush artifact, and larger sample size. Any biopsies in excess of those required by the investigator may be prepared as *shareable samples*. These will be lightly fixed in acetone, and embedded in GMA; an additional sample may be formalin fixed and embedded in paraffin, per protocols to be posted on the SARP web site at later date. Shareable sample biopsies will be retained en bloc, and should not be precut into sections.

It is recommended, if sufficient biopsy material exists, that sections be prepared at 2 μm thickness, 4 μm apart, and stained using monoclonal antibodies to recognize CD4, EG2, CD68, tryptase and neutrophil elastase positive cells (T-cells, activated eosinophils, macrophages, mast cells, and neutrophils). This information is useful to characterize the biopsy.

9.1.13 Postprocedure Follow-up and Monitoring

One hour minimum recovery time is recommended; two hours minimum recovery time is suggested if conscious sedation is used. Subjects with VSA will be hospitalized overnight, by protocol; thus, hospitalization in this group *does not* constitute a Serious Adverse Event. This requirement will be reconsidered after 6 months, in conjunction with the DSMB.

For subjects with severe or very severe asthma, albuterol 2.5 mg by nebulizer should be administered as soon as practical following the procedure. In subjects with very severe asthma, this dose is mandatory. In subjects with severe asthma, this dose may be omitted if all of the following are satisfied: the subject is symptom-free, not wheezing, and if tachycardia of more than 30 bpm above baseline heart rate is present. For other subjects, the administration of inhaled β-agonist following the procedure will be guided by clinical need, and the presence and degree of airflow limitation.

Assessment for the presence of hospitalization indicators (Section 9.1.14) should be made. If any are present, the subject should be hospitalized overnight. If the decision to hospitalize was not made prior to the procedure, the development of hospitalization indicators, and subsequent hospitalization, *does* constitute a serious adverse event.

Postbronchoscopy spirometry should be measured; for subjects other than VSA, the subject may be discharged following the minimum recovery time if FEV_1 reaches $\geq 85\%$ of that observed in the prealbuterol spirometry obtained prior to bronchoscopy (*Spirometry 2*).

Despite appropriate selection, preparation, procedural technique, postbronchoscopy monitoring, and physiologic assessment prior to discharge, it is possible that subjects occasionally will require urgent care following discharge from the procedure unit. Infrastructure should be in place to recognize the need for, and to facilitate, such care should the need arise.

Contact numbers must be provided to the subject. A follow-up call will be made to each subject with severe asthma or very severe asthma daily for three days. Other participants will receive one phone call the day after the procedure. In the unlikely event that postbronchoscopy concerns or issues have not resolved at the end of the scheduled contacts, ongoing contact and clinical care should be provided until the matter is appropriately resolved.

Adverse events should be documented. Serious unexpected adverse events, such as unexpected hospitalization or prolongation thereof, a life-threatening event, death, must be reported to the DCC and DSMB within 24 hours of the

investigator becoming aware of the event.

9.1.14 Hospitalization Indicators

Overnight hospitalization is mandatory for all subjects with VSA.

For any subjects who exhibit any of the following characteristics during or after bronchoscopy, overnight hospitalization should be provided:

- Significant cough persisting beyond 2 hours after completion of procedure
- Failure of PFTs after bronchodilator administration to return to within 15% of prebronchdilation FEV_1 at end of monitoring time
- Persistent hypoxia $< 90\%$ at end of monitoring time
- Persistent tachycardia > 130 bpm at end of monitoring time
- Unexpected altered mental status during or after procedure
- Significant hemoptysis > 50 ml
- Requirement for bronchodilator every 2 hours on more than 3 occasions
- In subjects with severe asthma, the unexpected absence of a companion or caretaker at home

Treatment should be directed towards resolving underlying airway obstruction and other pathophysiology, and relieving symptoms, based on the best clinical judgment of the physicians involved.

Follow-up telephone contact should be made for all subjects 24 hours after the procedure is completed. For subjects with SA and VSA, daily telephone contact for 3 days should be made. If issues have not resolved in either group at the time of the last scheduled contact, additional contact and necessary medical care should be arranged.

9.1.15 Maintenance and Equipment Matters

Consideration should be given to performing surveillance cultures of nominally sterile bronchoscopes on a periodic basis (3–6 month intervals) to ensure the adequacy of decontamination and sterilization procedures, consistent with local infection control policies. All relevant manufacturer recommendations about cleaning, maintenance, leak testing, and other issues should be followed.

9.2 Safety Algorithm

9.2.1 Part 1: Physiology

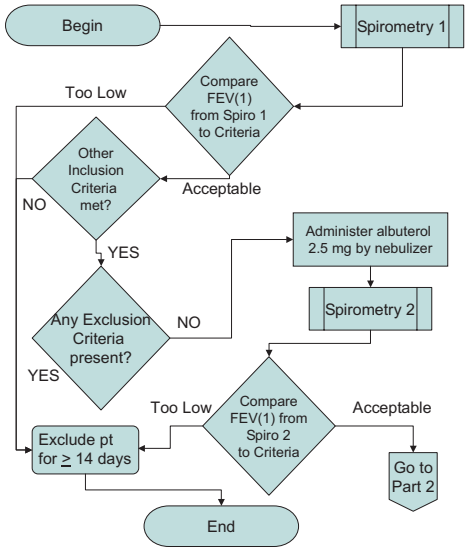

Figure 9.2: Bronchoscopy Safety Algorithm: Physiology. See Sections 9.1.3 and 9.1.4 for inclusion and exclusion criteria. See Table 9.3 for FEV_1 criteria.

9.2.2 Part 2: SA/VSA Determination

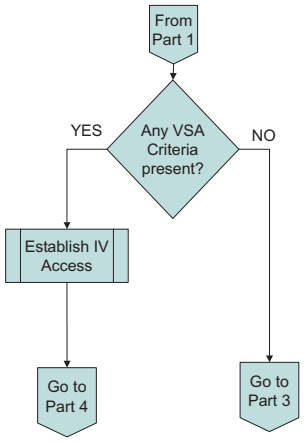

Figure 9.3: Bronchoscopy Safety Algorithm: SA/VSA Determination. See Section 9.1.3 for VSA criteria.

9.2.3 Part 3: IV Access in SA

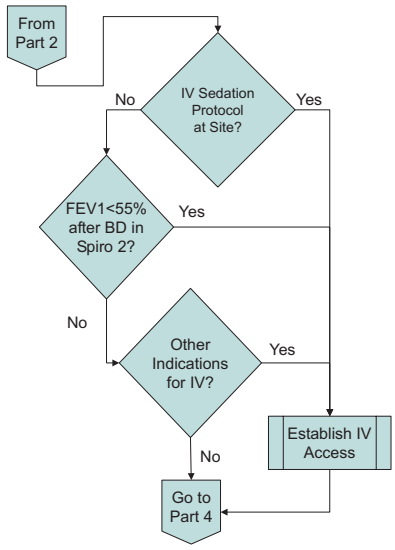

Figure 9.4: Bronchoscopy Safety Algorithm: IV Access in SA

9.2.4 Part 4: Procedure and Monitoring

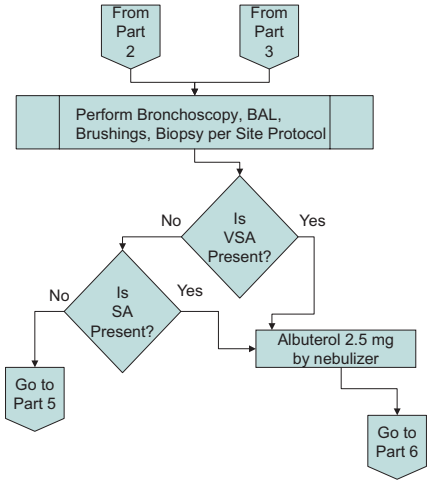

Figure 9.5: Bronchoscopy Safety Algorithm: Procedure and Monitoring

9.2.5 Part 5: Standard Care and Follow-up

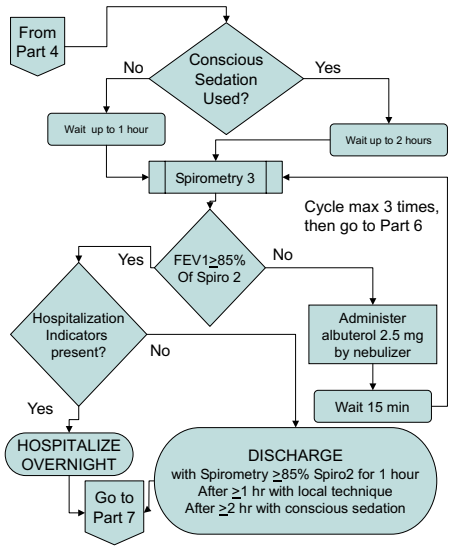

Figure 9.6: Bronchoscopy Safety Algorithm: Standard Care and Follow-up

9.2.6 Part 6: Higher Risk Care and Follow-up

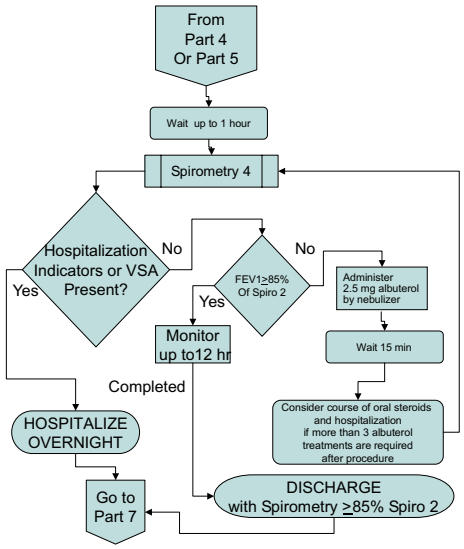

Figure 9.7: Bronchoscopy Safety Algorithm: Higher Risk Care and Follow-up

9.2.7 Part 7: Postbronchoscopy Follow-up

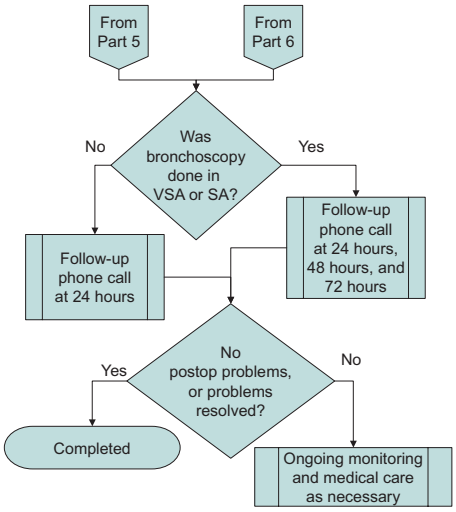

Figure 9.8: Bronchoscopy Safety Algorithm: Postbronchoscopy Follow-up

9.3 Pediatric Standards: 6–11 Years of Age

Children 6–11 years of age will not undergo bronchoscopy for research purposes. This section describes only the conditions under which samples obtained during a pediatric bronchoscopy performed exclusively for clinical purposes may be used and shared for research.

9.3.1 General Considerations

Children 6–11 years of age with severe asthma may require bronchoscopy for indications such as suspicion of foreign body, suspicion of infection, immotile cilia syndrome, congenital lesions of the airway, chronic aspiration, or persistent atelectasis. The indications for, timing of, anesthesia for, monitoring of, and recovery after these bronchoscopies are clinical decisions. General anesthesia or conscious sedation will be given to children 6–11 years of age; the physician who does the clinical bronchoscopy will determine the mode of anesthesia. Table 9.5 (p 127) lists common medications and dose ranges for these pediatric subjects. The guidelines for anesthesia and monitoring will be at least as stringent (generally substantially more stringent) than those for adults. Appropriate sites for doing bronchoscopy in pediatric subjects with severe asthma will be restricted to those with sufficient monitoring and staff suitable for the routine care of a child with a life-threatening respiratory illness.

9.3.2 Bronchoscopy Samples

Children aged 6–11 years will not have endobronchial biopsies for research purposes. The legal guardian(s) of 6–11-year-old children who undergo endobronchial biopsy for standard clinical indications and who meet SARP criteria will be asked to provide informed consent in order to share tissue with SARP investigators as follows:

- Standard clinical indications for endobronchial biopsy in children include diagnosis of immotile cilia syndrome, endobronchial tuberculosis, and histopathology of unknown endobronchial lesions.
- At least 6 tissue fragments will be submitted to the clinical pathology laboratory for fixation and analysis. Any tissue that remains after standard histopathological evaluation can be shared with SARP investigators.
- Specific contraindications to endobronchial biopsy are suspected vascular tumors of the airway (carcinoid), platelet count less than 50,000 mm^3 , and a known coagulation disorder.

In this way, tissue samples will be taken only when indicated for clinical diagnostic purposes.

9.3.3 Informed Consent

Two consents will be obtained. One will be the standard institution clinical procedure consent form. Under no circumstances will this consent form be obtained or signed by an investigator involved in the SARP. A separate consent, approved by the Institutional Review Board (IRB), will be obtained for using extra clinical specimens for research purposes. This consent will be obtained by a SARP-associated investigator. It will contain at least the following information:

- The procedure is not being performed for research purposes.
- The purpose of this study is to define what kind of chemical imbalances in the airway lead to severe asthma. The child is being included because he or she has asthma.
- Extra samples are not being obtained for research purposes.
- Standard procedures for lavage and, if indicated, biopsy will be used (if there is any extra fluid or tissue after adequate samples have been sent for clinical analysis, the parent or guardian consents for these to be used for research purposes).
- These samples may be shared with other centers in the SARP.

- The parent or guardian is free to choose not to participate in the study. Choosing not to participate will in no way alter the child's care.
- Because no additional procedures are being done for research purposes, participation does not add risk to the procedure.
- Information about participation in the study will be placed in the child's medical record. Officials of the Food and Drug Administration, local IRB, or NIH may have access to these records. Information may also be published, but the child will not be able to be identified in the publication.
- There is no specific benefit to the child's participation. The benefit to society is that childhood asthma may be better understood.
- There is no financial compensation for participation.

9.3.4 Unique Procedural Issues in Pediatrics

It will be preferred that standard bronchoalveolar lavage technique for asthma will be used. The bronchoalveolar lavage fluid should be warmed to 37°C. For children at least 12 years old, two 50-ml aliquots, pooled, will be ideal. For children less than 12, the aliquot volume will ideally be 1 ml/kg. The clinical bronchoscopist will determine how he or she wants the specimens used. Then, if there is any remaining specimen, the SARP investigator will be called and will have the option of triaging the sample to be processed as outlined in the adult protocol above, with the caveat that there will be much less sample and experiments of local relevance may take precedence.

9.4 Pediatric Standards: 12–17 Years of Age

9.4.1 Purpose

It is the primary goal of this document to provide collective experience and advice of the participating centers, in order to maximize the safety of pediatric subjects aged 12–17 years that participate as volunteers in the Severe Asthma Research Program. An important secondary goal is to facilitate the acquisition of airway samples (BAL fluid, BAL cells, bronchial brushings, and bronchial biopsies) that can be shared with other SARP investigators. This document is separate from the Standards proposed for adults and children 6–11 years of age.

Bronchoscopy in 12–17-year-old children will be performed for research purposes only or when clinically indicated and permission is obtained for additional research samples. It is our opinion that research studies in this age group are

needed to further our understanding of severe asthma. Conclusions which are simply extrapolated from adults, may be misleading. Therefore, in this group of 12–17-year-old children, the benefits of research bronchoscopy outweigh the potential risks.

9.4.2 General Considerations

Subjects must meet all inclusion criteria (Section 9.4.3) and not meet any exclusion criteria (Section 9.4.4) .

Considerations for all subjects

- Ability to cooperate with procedure
- Ability to give consent (parents of pediatric subjects) and assent (pediatrics)
- Clotting studies, CBC, and platelet counts may be done as a bronchoscopy screen at the discretion of investigators, but are not required.
- Coexisting lung disease

Considerations for asthmatic subjects

- Clinical stability, with no evidence of viral infection or exacerbation, consistent with the specific inclusion and exclusion criteria outlined below. A subject judged to be clinically unstable by the investigator or bronchoscopist should generally be excluded from bronchoscopy until acceptable clinical stability, as defined in the inclusion (Section 9.4.3) and exclusion (Sections 9.4.4 and 9.4.5) criteria, is achieved.
- Consideration should be given to overnight monitoring and observation in subjects with a history of frequent exacerbations (5 exacerbations in the previous 180 days)
- For all pediatric participants with severe asthma, a competent companion or caretaker should be available at home for at least 24 hours after the procedure. Identification of such a person should precede initiation of sedation and bronchoscopy.

Considerations about consent and assent

- In order to assure that participants and their parents have ample opportunity to review study related materials before the initial study visit, once a participant is scheduled for an initial visit, a packet of materials will be mailed to the participant's home. This packet will include the informed

consent document, the assent document, and other study-related information. The child and parent will have at least 48 hours to review this material in order to help them generate further questions they may have regarding study participation. They will be provided with a telephone number to call to ask any questions they have regarding the study and the consent and assent forms. In addition, a study investigator will call the family 24 hours before the visit to review the consent and assent forms and encourage any questions. The investigator will again be present to review the consent and assent forms at the time of the first visit and address any further questions or concerns.

- In addition to reviewing these documents, the investigator and study coordinator will reinforce that participation in the study is entirely voluntary. Participants may choose not to enroll, or they may withdraw at any time. Refusal to enroll or withdrawal will not affect the care they receive regarding their child's asthma.
- The investigator will discuss the risks and benefits with the child and will obtain written assent when required to do so by the local IRB.

9.4.3 Specific Inclusion Criteria

Subjects must meet each of the following criteria in order to qualify for the bronchoscopic study:

- PFT: Prebronchodilator FEV_1 in *Spirometry 1*, obtained before bronchodilator administration, must always be $\geq 45\%$ of predicted.
- PFT: Postbronchodilator FEV_1 in *Spirometry 2* obtained after administration of 2.5 mg albuterol by nebulizer must always be $\geq 55\%$ of predicted.
- Age: 12–17 years
- Gender: no selection criteria
- Minority status: no selection criteria
- No hospitalization for asthma in the previous 6 months and no intubation for asthma in the previous 12 months
- Subject must be judged otherwise to be clinically appropriate for bronchoscopy by the bronchoscopist. No specific PFT or other criteria, other than above, are used to document subject's clinical stability. Safety of the subject is the overriding concern in making this determination. Details of the clinical course must be detailed in the record.

9.4.4 General Exclusion Criteria

- Inability to provide informed consent (adult) or assent (pediatrics)
- Inability or unwillingness to complete schedule SARP procedures
- Lack of reliable communications channel (hard-wire phone, cell phone, email for follow-up contacts after bronchoscopy)

9.4.5 Specific Exclusion Criteria

Subjects with very severe asthma, as defined in the *Adult Bronchoscopy Standards* (Section 9.1), and subjects with a current asthma exacerbation are specifically excluded from pediatric bronchoscopy. Those experiencing an asthma exacerbation may be reevaluated in 14 days. The following exclusion criterion should exclude a subject from enrollment:

- More than 6 exacerbations in the previous 6 months
- Hospitalization for asthma in the previous 6 months
- Intubation for asthma in the previous 12 months
- Pulse oximetry on room air showing oxygen saturation of $< 90\%$
- Use of more than 8 puffs of a short-acting β-agonist per day
- In the presence of a regular long-acting β-agonist, the use of a short-acting β-agonist in excess of 4 puffs per day
- Regular corticosteroid dose of ≥ 20 mg/day
- Significant increase in asthma symptoms in the previous 48 hours, recognized as an increased use of a short acting β-agonist of more than 8 puffs/day over baseline
- A changed dose of oral corticosteroid (up or down) in the previous 14 days
- Current asthma exacerbation or other clinical instability in the judgment of the investigator or bronchoscopist
- If necessary, D_{LCO} can be performed at the discretion of the investigator to clarify the diagnosis of very severe asthma. In this setting, a D_{LCO} less than 70% predicted will be exclusionary. However, determination of D_{LCO} is not mandatory.
- The presence of clinically important comorbidities—these include uncontrolled diabetes, uncontrolled heart disease, acute or chronic renal failure, and uncontrolled hypertension—that increase the risk of significant adverse

events during bronchoscopy. The clinical judgment of the investigator or bronchoscopist is required, and in those cases in which judgments must be made, bias toward subject safety is essential.

9.4.6 Medication for Bronchoscopy

As the details of premedication are unlikely to affect shared samples, this aspect of the procedure is left to the discretion of the investigator and the bronchoscopist, as approved by the IRB of the investigator. Conscious sedation, delivered by IV or by mouth in a manner consistent with local guidelines on conscious sedation, will be given to all pediatric subjects 12–17 years of age.

Clinical judgment is critically important to select and administer conscious sedation. Dose titration is the rule rather than the exception, and fixed dosing regimens are neither commonly used clinically, nor biologically justifiable.

Table 9.5 lists common medications and dose ranges for pediatric subjects 6–17 years of age.

Table 9.5: Common Medications and Pediatric Dose Ranges

Class	Drug	Dose Range
Anxiolytic	Midazolam	0.025–0.050 mg/kg*
Anticholinergic	Atropine	0.01–0.02 mg/kg
	Glycopyrolate	8 μg/kg
β-adrenergic	Albuterol	1–5 mg
Narcotic-Antitussive	Demerol	1.0–2.2 mg/kg
	Fentanyl	1–2 μg/kg/dose†
	Codeine	10–20 mg
	Morphine	0.1–0.2 mg/kg

* Carefully titrate dose; the usual maximum dose is 10 mg, but as much as 0.4 mg/kg may be required.

† May repeat at 30-min intervals up to 4 μg/kg

9.4.7 Topical Anesthesia

As the details of topical anesthesia are unlikely to affect shared samples, this aspect of the procedure is left to the discretion of the investigator and the bronchoscopist. The spirit of this guideline is to minimize the lidocaine dose delivered to the volunteer. For purposes of calculation, all lidocaine administered is to be included, whether delivery is via gargle, aerosol, spray, or instillation. For

subject safety, the following guidelines on lidocaine dose are suggested:

> Lidocaine will be administered at a target dose range of 5–7 mg/kg over a 30-minute period. In unusual circumstances, however, a dose of up to 8–9 mg/kg may be required.

The amount of lidocaine used should be recorded in the procedure record sheet.

9.4.8 Procedural Monitoring

Because IV conscious sedation is employed, minimum monitoring includes

$$O_2 \text{ saturation, heart rate, BP, and ECG} \quad .$$

In addition, all local policies concerning conscious sedation should be observed.

All participants will receive supplemental oxygen (e.g., ≥ 2 liters/min) during the procedure. Persistent hypoxia of less than 92% oxygen saturation at end of monitoring will result in hospitalization; see Section 9.4.17 for all indicators of hospitalization.

9.4.9 Personnel Recommendations

To ensure subject safety and to facilitate appropriate processing of samples, the following minimum in suite personnel are recommended for all procedures:

- Bronchoscopist with pediatric experience
- Bronchoscopy assistant (a nurse, RT, or trained technician); this person will assist the bronchoscopist in monitoring the subject for signs of physiologic distress
- A second bronchoscopy assistant: an additional clinically trained individual in the bronchoscopy suite is recommended; this person's sole responsibility is to monitor vital signs, oximetry, and other physiological and clinical signs to ensure subject safety.
- Sample handler

9.4.10 Procedural Documentation

In all cases, documentation of details of procedure is required. Use of a site-specific standard form for all procedures is strongly encouraged in order to capture all relevant clinical issues; the details of the form will be institution-specific. An appropriate form is available from the DCC website. The following information should be captured:

Prior to Bronchoscopy

Mental status
Pulmonary examination
Vital signs
Baseline pulse oximetry
Investigator review of recent H&P and *SARP Prebronch Checklist*
Document 24/7-valid contact information provided to volunteer
Document that an adult caregiver will be available after the procedure and discharge

Note: For subjects for whom the bronchoscopist or the investigator determines an *a priori* need for overnight hospitalization and monitoring, *documentation of this decision before the procedure* is essential to avoid the requirement to report such hospitalization as a serious adverse event.

Procedural Details

Bronchoscopist and assistants
Premedications: dose and route
Topical anesthesia: type, dose
Route (oral or nasal)
Appearance of vocal cords
Sites of procedures (BAL, brushings, biopsies)
BAL: aliquot size, number of aliquots, total volume
Bronchial brushings: location
Biopsies: number and location

Procedural Monitoring

O_2 saturation, heart rate, BP, and ECG

Postprocedure

Document acceptable O_2 saturation on room air
Document FEV_1 within 15% of prebronchoscopy, prealbuterol value
Document 24/7-valid contact info provided to volunteer
Document determination of need for extended 12+ hour monitoring or hospitalization (see Sections 9.4.16 and 9.4.17)

9.4.11 Approach

The selection of transoral or transnasal approach will be left to the discretion and judgment of the bronchoscopist, based on individual subject characteristics, anatomy, choice of bronchoscope, and other local considerations. The approach route must be documented in the procedure record.

9.4.12 Bronchoalveolar Lavage: Performance

Bronchoalveolar lavage (BAL) will often be the first procedure performed (of BAL, brushings, and biopsies) because the latter two generate trauma which

will contaminate BAL fluids, even if the BAL is performed in a segment distinct from the brushings or biopsies. However, the needs and preferences of the individual investigators should be honored. If biopsies are performed before BAL, care must be taken to clear the working channel of the bronchoscope of contaminating blood and other debris.

Shareable samples of BAL should be obtained before bronchial brushings or bronchial biopsy—or taken from a different segment, and preferably a different lobe—in order to avoid contamination of the sample with blood.

In order to ensure maximal volume return, fluid to be used for BAL will be warmed to 37°C. Instillation of fluid will occur using hand pressure on a syringe, and the fluid will be recovered into the same syringe using hand suction to minimize mucosal trauma and contamination of BAL fluid with serum proteins.

BAL will employ 1 ml of saline per kg in weight for each aliquot, repeated up to 4 times (maximum 4 cc/kg). The standard technique will be instillation and recovery of 1 aliquot, followed by instillation and recovery of each of the remaining aliquots. If the recovered volume is < 15 ml from the first 100 ml of injectate, then the BAL will be stopped. The return from these syringes will be pooled, and the supernatant removed and frozen. The return from the first 2 aliqouts will be considered the standard *shareable sample* BAL material.

9.4.13 Bronchoalveolar Lavage: Processing

The return from the first 2 syringes will be pooled. No straining of fluid through gauze or wire mesh should be performed, in order to minimize loss of cells. Fluid is centrifuged to recover cell pellet. Excess supernatant is aliquoted to ten 1-ml samples (*shareable samples*). An additional 1-ml aliquot can be prepared with an antioxidant cocktail for the Cleveland site. An additional 1-ml aliquot mixed with an equal volume of ice cold methanol can be prepared for the Boston site.

The cell pellet is resuspended, cell counts are performed, and 12 cytocentrifuge slides are prepared. Two are stained with Diff-Quick or other histological stain and are interpreted for cellular differential. From this information, total and differential cell counts are computed. Of the other 10 slides, 5 are fixed in acetone, and 5 are fixed in 0.5% formalin; these latter 10 slides represent *shareable samples.*

9.4.14 Bronchial Brushings

In most circumstances, all required BAL procedures should be completed before brushings in order not to contaminate the BAL with red blood cells or other

brush-related traumatic artifacts.

Locale of brushing is at the discretion of the bronchoscopist, but typically it is done in a lower lobe segment, with 10 fore-and-aft strokes to dislodge epithelial cells. Cells can be dislodged by agitation in sterile media and recovered by centrifugation or by preparing a direct smear onto a sterile slide.

Epithelial cells in excess of those required for the project of the investigator may be used to prepare *shareable samples*. To do so, prepare 4 cytocentrifuge slides with 50,000 to 75,000 cells per 0.5 ml, or 200,000 to 300,000 cells total). Fix 2 in methanol. Fix 2 in 0.5% paraformaldehyde. An additional slide may be prepared by smearing the brush across a glass slide, and fixing with 0.5% paraformaldehyde (Cleveland site).

9.4.15 Bronchial Biopsy

Bronchial biopsy is performed after BAL. Specific locale is at the discretion of the bronchoscopist. Endobronchial biopsies should be performed under direct vision rather than blindly. The location from which biopsies are taken should be recorded in the procedure record. Transbronchial lung biopsy is a single-institution technique and will not be addressed herein.

Forceps should be sharp; disposable units are preferable. Alligator forceps are preferred to cup forceps due to less crush artifact and larger sample size.

Any biopsies in excess of those required by the investigator may be prepared as *shareable samples*. These will be lightly fixed in acetone and embedded in GMA; an additional sample may be formalin-fixed and embedded in paraffin according to protocols that will be posted on the SARP website at a later date. Shareable-sample biopsies will be retained en bloc, and should not be precut into sections.

It is recommended, if sufficient biopsy material exists, that sections be prepared at 2 μm thickness, 4 μm apart, and stained using monoclonal antibodies to recognize CD4, EG2, CD68, tryptase, and neutrophil elastase positive cells (T-cells, activated eosinophils, macrophages, mast cells, and neutrophils). This information is useful to characterize the biopsy.

9.4.16 Postprocedure Follow-up and Monitoring

Two-hour minimum recovery time is recommended after conscious sedation. For those subjects with a history of exacerbation within 30 days, monitoring up to 12 hours by trained personnel—under the supervision of, with immediate access to—a physician is recommended.

The administration of inhaled β-agonist after the procedure will be guided by clinical need as well as the presence and degree of airflow limitation.

Assessment for the presence of hospitalization indicators (Section 9.4.17) should be made. If any indicators are present, the subject should be hospitalized overnight. If the decision to hospitalize was not made before the procedure, the development of hospitalization indicators and the subsequent hospitalization constitutes a serious adverse event.

Despite appropriate selection, preparation, procedural technique, postbronchoscopy monitoring, and physiological assessment before discharge, it is possible that subjects occasionally will require urgent care following discharge from the procedure unit. Infrastructure should be in place to recognize the need for, and to facilitate, such care should the need arise.

Postbronchoscopy spirometry must be measured: a subject may be discharged if FEV_1 reaches $> 85\%$ of that observed in the prealbuterol spirometry obtained before bronchoscopy (*Spirometry 2*).

In the unlikely event that postbronchoscopy concerns or issues have not resolved at the end of the scheduled contacts, on-going contact and clinical care should be provided until the matter is appropriately resolved.

Adverse events should be documented. Serious unexpected adverse events, such as unexpected hospitalization or prolongation thereof, a life-threatening event, or death must be reported to the DCC and to the DSMB within 24 hours of the investigator learning of the event.

Contact numbers must be provided to the subject. Follow-up telephone calls will be made to each participant once a day for 3 days after the procedure.

9.4.17 Hospitalization Indicators

Consideration for overnight hospitalization should be given for subjects who exhibit any of the following characteristics during or after bronchoscopy:

- Significant cough persisting beyond 2 hours after completion of procedure
- Failure of PFTs to return to within 15% of prebronchodilation FEV_1 at end of monitoring time
- Persistent hypoxia $< 92\%$ at end of monitoring time
- Persistent tachycardia > 130 bpm at end of monitoring time
- Unexpected altered mental status during or after procedure
- Significant hemoptysis > 25 ml

- Unexpected absence of a companion or caretaker at home

Treatment should be directed toward resolving underlying airway obstruction and other pathophysiology and relieving symptoms, based on the best clinical judgment of the physicians involved.

9.4.18 Maintenance and Equipment Matters

Consideration should be given to performing surveillance cultures of nominally sterile bronchoscopes on a periodic basis (3–6 month intervals) to ensure the adequacy of decontamination and sterilization procedures, consistent with local infection control policies.

All relevant manufacturer recommendations about cleaning, maintenance, leak testing, and other issues should be followed.

9.5 Stopping Rules

9.5.1 Purpose

This section codifies policies for imposing a temporary moratorium on the conduct of bronchoscopies or specific bronchoscopic procedures at one or more of the SARP sites. These policies are designed to maximize subject safety and to minimize the occurrence of adverse events and serious adverse events. A moratorium can be general (implemented for all bronchoscopies and related procedures) or limited (implemented for a specific procedure: e.g., bronchoalveolar lavage, bronchial biopsy, or transbronchial biopsy).

There are specific stopping rules for transbronchial biopsy, a single-center procedure; these stopping rules are detailed in Section 9.6.4 (p 140) and are hereby incorporated by reference.

9.5.2 Existing Information about Adverse Events

In general, the current literature fails to establish the expected incidence of adverse events related to bronchoscopy. In published reports, there is considerable heterogeneity in patient populations studied, invasive procedures employed, and the vigor and thoroughness with which investigators identified adverse events. At present, there is insufficient experience within the SARP to provide accurate estimates of the expected frequencies of AEs and SAEs.

The SARP will use an aggressive approach to recognize and identify adverse events. We will use a process-driven approach to analysis and response to ad-

verse events that may occur during the SARP. Below, we define the process by which the Steering Committee will evaluate and respond to AEs and SAEs, and we propose 3 types of moratoria, and we describe the process by which moratoria are implemented and lifted.

9.5.3 Review of All Adverse Events

Each principal investigator is scientifically and ethically responsible for monitoring the occurrence of adverse events and severe adverse events at their site or center. If significant concerns about AEs or SAEs arise at a particular site or center, a local moratorium should be considered. In addition, the SARP Steering Committee, during its routine monthly telephone or in-person meetings, will review summary statistics on the occurrence of AEs and SAEs. After review, summary statistics and supporting details will be forwarded to the DSMB, along with a recommendation for specific action, if any action is deemed necessary.

On the occurrence of any SAE, the center or site will notify the DCC within 2 working days. The DCC will arrange for an urgent phone conference of a quorum of the Steering Committee to be held within 2 working days for discussion of the SAE. The recommendation for action of the Steering Committee from this phone conference as well as the details of the SAE will be forwarded to the DSMB for its review.

In addition, the SARP Steering Committee and DSMB will conduct an ongoing review and analysis of AE and SAE reports submitted by each site or center, and they will consider collectively data from the entire SARP. If the Steering Committee and DSMB are concerned about the results of such an analysis, they can impose one of 2 moratoria:

1. *a site- or center-specific moratorium* , if the review suggests that the matter is limited to one or more specific sites or centers, or

2. *a program-wide moratorium* , if the review suggests that the matter could be reasonably expected to affect all sites and centers in the SARP.

9.5.4 Types of Moratoria

Investigator-Initiated: Site- or Center-Specific

If an investigator learns of a local matter that could compromise subject safety or could increase the risk of an AE or SAE, they should impose a local, site- or center-specific moratorium on bronchoscopic procedures. An example of this moratorium would be the recognition of improper cleaning procedures for the

bronchoscopes, faulty procedures that contribute to an AE or SAE; the moratorium would be lifted after appropriate changes had been implemented.

Although the issue that triggers an investigator-initiated moratorium may involve other sites or centers, the moratorium does not impact formally other sites or centers. Nevertheless, each principal investigator should ascertain whether the circumstances that led to the moratorium are relevant at their site.

Steering Committee or DSMB-Initiated: Site- or Center-Specific

If the ongoing safety review by the Steering Committee or DSMB reveals an matter of concern that is likely or clearly related to a single site or center, the Board can impose a site- or center-specific moratorium on all procedures related to bronchoscopy. An example of this moratorium would be an unacceptably high rate of significant bleeding after bronchial biopsy at a single site or center; the moratorium would be lifted after the reasons were identified and corrective action taken.

Steering Committee or DSMB-Initiated: Program-Wide

An important component of adverse event monitoring is the identification of AEs, SAEs, and other events that may happen rarely at an individual site but happen more frequently across all SARP sites. If the collective incidence concerns the Steering Committee or the DSMB, a program-wide moratorium could result. In addition, an adverse event of major consequence—serious injury or death—could result in a program-wide moratorium imposed by the Steering Committee or DSMB.

9.5.5 Guidelines for Imposing a Moratorium

Investigator-Initiated: Site- or Center-Specific

A principal investigator should consider imposing a site- or center-specific moratorium if the following occurs:

- The principal investigator learns of a situation at their site or center that could compromise subject safety or that could increase the risk of an AE or SAE.

Within 3 working days, the principal investigator will notify the DCC of the moratorium; the DCC will notify the Steering Committee. A committee composed of the Chair of the Steering Committee, a representative from the DCC, and a representative from the NIH will determine the level of urgency of

the matter, the breadth of applicability to SARP, and the appropriate action: a referral to the DSMB, an urgent conference call of the Steering Committee, or an agenda item for the next scheduled Steering Committee conference call.

Steering Committee or DSMB-Initiated: Site- or Center-Specific

The Steering Committee or DSMB should consider imposing a site- or center-specific moratorium if either of the following occur:

- An equipment-related concern arises. In this case, the moratorium would apply to each site at which the equipment of concern is used.
- An analysis by the Steering Committee or DSMB demonstrates that AEs or SAEs occur at a single site or center.

The DSMB will notify the DCC, the Steering Committee, and the relevant investigators of the moratorium.

Steering Committee or DSMB-Initiated: Program-Wide

The Steering Committee or DSMB will impose immediately a program-wide moratorium if either of the following occur:

- Any death is related to, or likely related to, a SARP bronchoscopy or related procedure, or
- Cardiopulmonary instability or unexpected endotracheal intubation is related to, or likely related to, a SARP bronchoscopy or related procedure.

In this situation, the center must notify the DCC within one working day; the DCC will immediately notify the DSMB and the entire SARP that a program-wide moratorium has been imposed.

9.5.6 Summary of Monitoring and Responses to Adverse Events

Adverse Events

Each month, the DCC will prepare a summary of adverse events; this summary will be a formal agenda item on each monthly Steering Committee conference call. The summary along with any related Steering Committee concerns and recommendations will be forwarded to the DSMB for review and action.

Serious Adverse Events

Serious adverse events are defined in the Federal Register. Serious adverse events expected to occur occasionally as a result of bronchoscopy are outlined in the

Manual of Procedures and consent form; unexpected serious adverse events may also occur. Each month, the DCC will prepare a summary of serious adverse events; this summary will be a formal agenda item on each monthly Steering Committee conference call. The summary along with any related Steering Committee concerns and recommendations will be forwarded to the DSMB for review and action.

A serious adverse event related temporally to bronchoscopy will be reported to the DCC within 2 working days. The Steering Committee will convene by telephone within 2 working days after the DCC broadcasts the bronchoscopy-related adverse event; the Steering Committee will review details of the serious adverse event, prepare a response and recommendation, and subsequently forward all relevant information to the DSMB. A quorum of the Steering Committee is defined as representation by the Principal Investigator or a Coinvestigator from at least 5 of the 8 centers.

Severe Adverse Events

Death or significant cardiopulmonary instability with or without the need for endotracheal intubation related to bronchoscopy or one of its associated procedures, will be reported within 1 working day to the DCC, which will forward the information immediately to the DSMB and notify the entire SARP. A 48-hr program-wide moratorium will begin when the investiagtors receive notifcation from the DCC.

9.5.7 Procedure for Lifting a Moratorium

A moratorium may be lifted only after DSMB review of the detailed descriptions of the adverse events or severe adverse events and descriptions of the changes proposed to minimize the likelihood of their recurrence. In most cases, a change in procedures or a change in subject-selection criteria will be appropriate; the scope of these changes could be site-specific or program-wide.

9.6 Transbronchial Biopsies

9.6.1 Background

Considerable data support the presence of inflammatory and remodeling processes of the distal lung in asthma, but specific pathologic studies are limited. There are 3 published transbronchial biopsy studies—one in nocturnal asthma

and 2 in severe asthma—that suggest there is increased and altered inflammation in the distal lung.[2]

In addition, there appears to be a different response to inhaled medications in severe asthmatic patients, a response that might result from limited ability of these drugs to reach the distal lung. Therefore, studies of the distal lung could have considerable impact on treatment options for patients with severe asthma. In SARP, we propose to evaluate parenchymal-airway attachments in asthmatic subjects to determine whether abnormalities in elastin or myofibroblasts exist in the distal lung. These changes might modify contractility and collapsibility of the airways and might explain the loss of elastic recoil, phenomena that have been observed in patients with severe asthma. In SARP II, only the University of Pittsburgh will perform transbronchial biopsies to evaluate parenchymal-airway attachments.

For more than 10 years, Dr. Wenzel, now at the University of Pittsburgh, has done transbronchial biopsies in subjects with severe asthma for research purposes: nearly 100 bronchoscopies, resulting in 200–400 transbronchial biopsies, have been performed. During all these biopsies, just one small pneumothorax—10% of lung volume—occurred; the pneumothorax resolved with supportive, noninvasive management. There have been no episodes of clinically significant bleeding. Usually, coughing from transbronchial biopsy is less than with lavage. When endobronchial biopsy, transbronchial biopsy, and bronchoalveolar lavage are all done, the total length of the procedure is less than 30 minutes. Therefore, we propose that the risk-benefit ratio is acceptable in asthmatic subjects.

9.6.2 Inclusion and Exclusion Criteria

General inclusion and exclusion criteria for endobronchial biopsies follow those outlined in the *Bronchoscopy Standards*, Sections 9.1.3 (p 106) and 9.1.4 (p 109), of the *Manual of Procedures.* For ethical reasons, transbronchial biopsies will not be done in healthy control subjects.

Inclusion Criteria for Subjects with Severe Asthma

- PFT: Prebronchodilator FEV_1 must be $> 50\%$ of predicted
- PFT: Postbronchodilator FEV_1 must be $> 60\%$ of predicted
- Age: 18–55 years
- Clinically stable according to endobronchial biopsy criteria

[2] Am J Respir Crit Care Med 1996; 154: 1505–1510; Am J Respir Crit Care Med 1997; 156: 737–743; Eur Respir J 2002; 20: 254–259.

- Able to withhold bronchodilator for 4 hours: because bronchodilator will not be withheld before the procedure, this criterion is for safety only
- Undergoing a clinically indicated bronchoscopy
- Normal INR and platelet count

Inclusion Criteria for Subjects with Mild-to-Moderate Asthma

- PFT: Prebronchodilator FEV_1 must be $> 55\%$ of predicted
- PFT: Postbronchodilator FEV_1 must be $> 70\%$ of predicted
- Age: 18–55 years
- Clinically stable
- Normal INR and platelet count

9.6.3 Procedure

The preparation for transbronchial biopsies will follow the general principles outlined for endobronchial biopsies. Additional precautions such as the use of fluoroscopy, time of the procedure, limits to the procedure, and the requirement that 2 physicians be present throughout the procedure will be taken to ensure subject safety.

To optimize lung function, all studies will be done after 10 AM . Biopsies will be done using fluoroscopic guidance. In general, biopsies will be taken roughly 3 cm from the fluoroscopically imaged chest wall to enhance the ability to obtain small airway tissue.

The first 10 subjects in each group (severe and mild-moderate) will undergo only 2 procedures: transbronchial biopsy with endobronchial biopsy in the subjects with severe asthma and transbronchial biopsy with either endobronchial biopsy or lavage in the mild-moderate subjects, depending on the specific nature of the scientific protocol.

If we can demonstrate that 2 procedures are safe, we hope to do all 3 procedures—endobronchial biopsy, lavage, and transbronchial biopsy—in both groups of subjects in the future.

Four transbronchial biopsies will be taken during each procedure for subsequent research analysis. This is 30–50% of the standard number taken in diagnostic and interstitial lung disease studies. The smaller number of biopsies will lessen the incidence of bleeding and pneumothorax; in published studies, the

rate of pneumothorax ranges from 1–5%. Epinephrine is mixed and available if excessive bleeding occurs.

Two experienced physicians, a registered nurse, respiratory therapist, and research technician will be present during each transbronchial procedure.

The time of radiation exposure will be recorded during all studies to estimate radiation dose during each procedure. Current Pittsburgh equipment is expected to expose a subject to 3–4 mSv of radiation; this amounts to 9–12 months of background radiation in Pittsburgh. This dose has been approved by the University of Pittsburgh Institutional Review Board.

9.6.4 Monitoring

Chest x-rays will be done immediately after the procedure and after any change in symptoms. Initially, all transbronchial biopsy subjects will be monitored overnight in the GCRC. Postbronchoscopy lung function will be monitored and treated using the same criteria as outlined in the *Bronchoscopy Standards*. In the absence of a severe adverse event, we will submit to the DSMB a detailed safety report within 7 days of biopsy for each of the first 3 subjects.

The DCC, NIH, and DSMB will be notified within 24 hours of any significant bleeding (>50 ml) or pneumothorax, including small ones that do not require drainage. We will file detailed reports on the first 7 subjects and propose—if these subjects experience no problems—that consideration be given to including subjects with lower lung function.

Unless there is a larger-than-expected dose in the first several subjects, the dose of radiation received by the subjects will be recorded and, after 7 subjects, this information will be submitted to the DCC, NIH, and DSMB.

Stopping Rules

- Any death from transbronchial biopsy
- More than one pneumothorax in the first 10 subjects
- More than one episode of significant bleeding in the first 10 subjects

9.6.5 Consent Language

Bronchoscopy with Transbronchial Biopsy

As part of the bronchoscopy described above, small pieces of tissue can also be removed from the parts of your lung beyond the visual limits of the bronchoscope. This procedure will enable small pieces of tissue to be gathered from

the smaller airway and the alveolar sacs (known as the distal lung). This part of your lungs (the smaller airways and the alveolar sacs) is different from the large airways where the biopsies were taken for the first part of the procedure. To obtain biopsy tissue from the distal lung, the study has to be done in the radiology (x-ray) department so that the instrument used to do the biopsies can be seen on the x-ray. When the bronchoscopist determines that the instrument is in the distal region of the lungs, a small piece of tissue is removed. Four biopsies will be done for research puposes. [*Note*: This section is for asthmatic subjects older than 18 years of age.]

Risks

The risks of transbronchial biopsy are similar to those outlined above (see endobronchial biopsy), but in addition, include the risk of a pneumothorax, or collapsed lung. This occurs when the biopsy is taken too close to the envelope that surrounds the lung (the pleura), such that it is punctured. This causes the air to escape from the lungs into that space around the lungs, 'collapsing' the lung to various degrees. This occurs approximately 1–2% of the time. Most of the time, the air leak is small and you will be treated with oxygen and observation only. However, it is also possible that the collapse of the lung will be severe enough that it will require immediate treatment to remove the escaped air. The collapsed lung would be treated by inserting a small tube (a chest tube) between your ribs, to remove the air. A local anesthetic will be given prior to the insertion of the chest tube. This tube would likely remain in place for about 24 hours, and you would be hospitalized overnight (sometimes longer) at University Hospital. Rarely, a surgical procedure is needed to sew up the hole.

Occasionally, 1–2% of the time, there can be significant bleeding (more than 3 tablespoons) from a transbronchial biopsy. However, blood tests will be done before the test to screen for any abnormal bleeding problems.

Radiation Exposure

You will be exposed to x-ray radiation during the bronchoscopy. A chest x-ray will be taken after the procedure to make sure you do not have a collapsed lung. The amount of radiation from a fluoroscopy (the x-ray machine) used during the bronchoscopy is about 3 mSv per bronchoscopy. This is the same as the amount of normal background radiation exposure a person receives in 1 year living at sea level or in 9 months living in Colorado. The more radiation you

receive over your lifetime, the greater the risk of having cancerous tumors or of causing changes in genes. Changes in genes might cause abnormalities or disease in your future offspring. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risks is not known. Women who are or could be pregnant should not participate in this study nor should they receive unnecessary radiation. If you suspect that you are or may be pregnant, please tell the study personnel immediately. A lead shield will be placed (waist to mid-thigh) over the reproductive organs to protect these organs from exposure.

9.7 Approval to Proceed with Bronchoscopy

In order to ensure prompt collection and submission of data to the Data Coordinating Center, each Center must contact the DCC before it bronchscopes a given subject. When a Center provides the subject ID to the DCC, the DCC confirms that data from these forms are in the SARP database:

- Asthma Quality of Life Questionnaire
- Screening Questionnaire for Severe Asthma
- Atopic Diseases
- Demographic Information
- Environmental Factors
- Family History
- General Symptoms of Lung Disease
- Medical History
- Medication History
- Provoking Factors of Asthma and Lung Disease
- Smoking History
- Maximum Postbronchodilator Value of Lung Function

If all forms for the subject have been submitted to the DCC, the DCC relays approval to proceed with bronchoscopy to the Center. If one or more form is not in the database, the DCC identifies the forms(s) that must be submitted before the bronchoscopy can proceed.