Monitoring of noninvasive ventilation by built-in software of home bilevel ventilators: a bench study

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Monitoring of Noninvasive Ventilation by Built-in Software of Home Bilevel Ventilators

A Bench Study

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Background: **Current bilevel positive-pressure ventilators for home noninvasive ventilation (NIV) provide physicians with software that records items important for patient monitoring, such as compliance, tidal volume (V_T), and leaks. However, to our knowledge, the validity of this information has not yet been independently assessed.**

Methods: **Testing was done for seven home ventilators on a bench model adapted to simulate NIV and generate unintentional leaks (ie, other than of the mask exhalation valve). Five levels of leaks were simulated using a computer-driven solenoid valve (0-60 L/min) at different levels of inspiratory pressure (15 and 25 cm H_2O) and at a fixed expiratory pressure (5 cm H_2O), for a total of 10 conditions. Bench data were compared with results retrieved from ventilator software for leaks and V_T.**

Results: **For assessing leaks, three of the devices tested were highly reliable, with a small bias (0.3-0.9 L/min), narrow limits of agreement (LA), and high correlations (R^2, 0.993-0.997) when comparing ventilator software and bench results; conversely, for four ventilators, bias ranged from −6.0 L/min to −25.9 L/min, exceeding −10 L/min for two devices, with wide LA and lower correlations (R^2, 0.70-0.98). Bias for leaks increased markedly with the importance of leaks in three devices. V_T was underestimated by all devices, and bias (range, 66-236 mL) increased with higher insufflation pressures. Only two devices had a bias < 100 mL, with all testing conditions considered.**

Conclusions: **Physicians monitoring patients who use home ventilation must be aware of differences in the estimation of leaks and V_T by ventilator software. Also, leaks are reported in different ways according to the device used.** ***CHEST 2012; 141(2):469–476***

Abbreviations: IPAP = inspiratory positive airway pressure; NIV = noninvasive ventilation; PEEP = positive end-expiratory pressure; V_T = tidal volume

Noninvasive ventilation (NIV) is widely accepted as a long-term treatment of chronic hypercapnic respiratory failure related to restrictive disorders and, although to a lesser degree, COPD. NIV aims to correct symptoms of nocturnal hypoventilation, improve arterial blood gases, prevent the evolution toward secondary pulmonary hypertension and cor pulmonale, and improve quality of life and quality of sleep. Home ventilators have evolved rapidly since the first cohort studies, with an increasing use of pressure-cycled bilevel ventilators over the past 20 years.[1]

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Home NIV is widely used, according to a 2005 report summarizing data on > 27,000 users in 16 European countries.[2] Monitoring of the efficacy of long-term NIV usually relies on medical history, daytime arterial blood gases, and nocturnal pulse oximetry, sometimes coupled with transcutaneous capnography.[3] An increasing awareness of nocturnal respiratory events occurring under NIV in the past several years has led to a wider use of respiratory polygraphy and polysomnography to better understand, define, and detect these events.[4-7] The most frequent problems detected are unintentional leaks, patient-ventilatory asynchrony, and obstructive or central events (either residual or induced by NIV), which have been recently extensively reviewed.[6] The latest generations of home ventilators have built-in software that provides the physician with potentially valuable information such as compliance, estimation of leaks, tidal volume (VT), minute ventilation, respiratory rate (RR), percentage of inspirations triggered by the patient, percentage of pressurizations interrupted by the patient (cycling), and indices of apnea and/or apnea-hypopnea. However, to date and to our knowledge, the only study to explore the validity (reliability) of this information in one home ventilator is that of Rabec et al.[8] Because previous bench tests have shown high variability in the performance of commercialized home ventilators,[9-16] we undertook the present study to assess the reliability of (1) the quantification of leaks and (2) the estimation of VT in a panel of home ventilators equipped with built-in monitoring software commercialized in Western Europe.

Materials and Methods

A panel of home ventilators distributed in Western Europe and equipped with built-in software providing at least monitoring of leaks, RR, and VT were tested. Seven ventilators were included: Monnal T30 (Airox), Synchrony and Trilogy (Philips Respironics), Ventimotion (Weinmann), Vivo 40 (Breas), and VPAP III ST and VPAP IV ST-A (ResMed). Software brands and the modes of estimation of leaks are summarized in Table 1. Tests were performed between July and September 2009.

NIV Bench Model

The bench model, illustrated in Figure 1, was derived from a bench test used in previous studies from our group[9,17-19] and adapted to simulate NIV conditions with and without unintentional leaks (defined as leaks other than those generated by the exhalation valve of the mask or tube). The model consists of a paired bellows system (PneuView AI 2601I TTL; Michigan Instruments). One chamber is connected to an ICU ventilator (Evita 4; Drägerwerk AG), which is set in pressure control mode to mimic patient inspiratory effort. The two chambers are linked together by a rigid metal strip; therefore, inflation of the first chamber inflates the second (the "patient" chamber), which is connected to the tested ventilator. Hence the onset of passive inflation is detected as an "inspiratory" effort by the tested device, which in turn triggers a pressure-support response.

Table 1—*Home Ventilators Tested, Compatible Software, and Modality of Estimation of Leaks*

Monitoring Ventilator	Software	Leaks
Monnal T30	Bora Soft V.6	Average leak[a]
Synchrony	Encore Pro 2	Average leak[a]
Trilogy	Direct View	Average leak[a]
Ventimotion	Ventisupport	Average leak[a]
Vivo 40	Vivo PS Software 3	Average leak at expiratory positive airway pressure[b]
VPAP III ST	ReScan 3.10	Average leak without intentional leaks[c]
VPAP IV ST-A	ReScan 3.10	Average leak without intentional leaks[c]

All ventilator software provides respiratory rate.
[a]Value provided is average leak during whole respiratory cycle and includes intentional and unintentional leaks.
[b]Value provided is average leak at the expiratory positive airway pressure value only and includes intentional and unintentional leaks.
[c]Value provided is average leak during the whole respiratory cycle and includes unintentional leaks only (intentional leaks are estimated based on type of mask and pressure settings).

The model allows the adjustment of elastance and airway resistance, which for this study were set at "normal values."[9]

To simulate NIV conditions, a PVC head model (Bill I; VBM Medizintechnik GmbH) equipped with an "upper airway" and a "trachea" was used. The tested ventilator was connected to the head by an adult medium-size oronasal NIV mask with an intentional leak (Ultra Mirage Full Face Mask; ResMed), fitted to the mask with its own elastic headgear.[18,19] A "Y" piece was introduced between the ventilator and the full-face mask. The second limb was connected to a solenoid valve generating predetermined leaks. Flow sensor transducers (Biopac Systems Inc) were placed between the "trachea" and "lung compartment" of the "patient" (Fig 1, FS_1), between the tubing and the mask (Fig 1, FS_2), and on

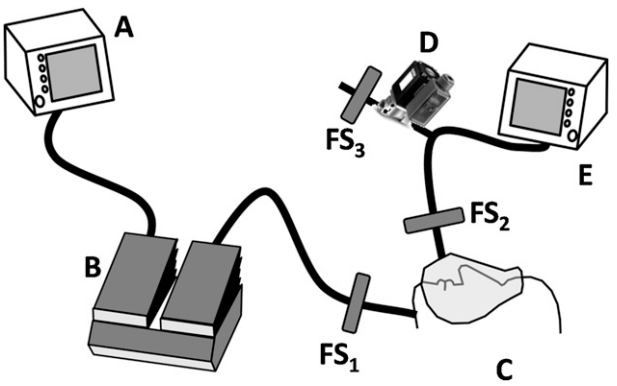

Figure 1. Diagram shows organization of bench study. A, driving ventilator. B, double bellows; one compartment is inflated by the driving ventilator and drives the second compartment, generating an inspiratory effort. C, head model with face mask connected to test ventilator and "trachea" connected to "patient compartment" of bellows. D, solenoid valve generating calibrated unintentional leaks commanded by computer. E, test ventilator. FS_1, flow sensor that measured airflow effectively delivered to "patient's" airways ("trachea"). FS_2, flow sensor that measured airflow delivered to the head mask by the ventilator (airflow generated by ventilator to unintentional leak). FS_3, flow sensor that measured airflow through the "unintentional leak valve." All flow sensors were connected to the data acquisition system.[9,17-19]

the "Y" tubing, after the leak generator (Fig 1, FS_3). All measurements were performed at an FIO_2 of 0.21. Data were stored on a laptop via an analog-digital converter (MP100; Biopac Systems Inc) sampled at 200 Hz, and stored in a laptop computer for subsequent analysis (Acknowledge software; Biopac Systems Inc).

Unintentional Leaks

A leak generator was developed for this study by the Engineering School of Geneva (HEPIA). The system included a solenoid valve controlled by a portable computer (Microsoft Visual Basic Visual Basic 6.0; Microsoft Corporation) that could be opened from 0 to 10 mm, thus generating a maximal unintentional leak of 60 L/min at 25 cm H_2O of inspiratory positive airway pressure (IPAP). Five levels of leaks were simulated (0, 10, 24, 40, and 60 L/min) at two different levels of inspiratory pressure (15 and 25 cm H_2O). The leaks were monitored by the flow sensor (Fig 1, FS_3).

Measured Parameters

Bench Data: Each condition was recorded during 10 min, and all cycles were taken in account. For each recording, the following items were recorded: VT, RR, mask ("intentional") leak, and induced ("unintentional") leak. VT was calculated by the integration of "trachea" flow (Fig 1, FS_1) during the recording and divided by the number of pressurizations.

The flow sensors (Fig 1, FS1-3) made it possible to analyze (1) airflow effectively delivered to the head mask airways ("trachea") (Fig 1, FS1); (2) airflow delivered to the "patient" by the ventilator (Fig 1, FS2), which corresponds to the airflow generated by the ventilator minus the unintentional leak; and (3) airflow through the "unintentional leak valve" (Fig 1, FS3).

Mask leaks (referred to as "intentional leaks") were derived by subtracting the "trachea" flow from the ventilator flow (mask leaks = $FS_2 - FS_1$). Induced leaks were derived from the integral of the flow curve obtained by the sensor situated just after the leak generator valve (Fig 1, FS_3).

Measurements performed using the Ultra Mirage Full Face Mask, with and without the built-in exhalation valve, allowed us to estimate unintentional leaks around the mask at approximately 0.4 L/min, which was considered negligible.

Ventilator Software Data: Data on leaks, VT, and RR were collected for each home ventilator from the provided software.

Experimental Protocol

Recordings were performed with the bench model simulating normal respiratory mechanics (elastance, 20 cm H_2O/L; resistance, 5.6 cm H_2O/L/s).

Driving Ventilator

The driving ventilator was set in pressure-controlled mode with the following settings: inspiratory pressure, 10 cm H_2O > positive end-expiratory pressure (PEEP); PEEP, 5 cm H_2O (ie, simulated inspiratory effort, 10 cm H_2O); pressurization slope (rise time), 200 μs; duration of inspiration, 1 s; and RR, 12 cycles per min.[9]

Tested Ventilator

The tested ventilator was set in pressure-support mode, named differently according to different tested ventilators. The IPAP was set at 15 and 25 cm H_2O, and PEEP was set at 5 cm H_2O. The inspiratory trigger and cycling criterion, when adjustable, were set at default values. The pressurization slope was set at its steepest value. The back-up frequency for each device was set at 10 cycles per min.

Statistical Analysis

All results are expressed as mean values. Because values were obtained during a bench test, they were considered stable and the standard deviation was considered negligible. The study protocol included testing at two levels of IPAP and five levels of "unintentional" leaks, for a total of ten conditions.

The agreement between parameters assessed simultaneously by bench test measurements and ventilator software data (leaks and VT) was expressed as proposed by Bland and Altman,[20] thus reporting for each ventilator the bias (average difference between measurements), and the limits of agreement (95% CI for the difference between measurements [ie, $1.96 \times SD$])(Fig 2) (Tables 2, 3).

Values obtained for VT and leaks through bench test measurements and ventilator software data were compared by the means of a linear regression (Pearson coefficient), and results of R^2 were reported to quantify the percentage of the variance of one parameter explained by the other. To quantify the influence of IPAP and leaks on estimation of VT, we performed a multivariate linear regression analysis. A value of $P < .05$ was considered significant.

Results

Leaks

Figure 2 and Table 2 summarize results regarding leaks. For ventilators F and G, intentional leaks (ie, of mask exhalation valve) are not included in data reported by the ventilator software, and therefore were subtracted from total leaks measured by the bench test to allow comparison between the bench test and software values. With this kept in mind, the threshold of clinical relevance for leaks suggested, arbitrarily, by manufacturers is 24 L/min for devices F and G.[8,21] For other devices, these limits are not specified, and the estimation of leaks should take into account pressure levels and types of mask.[22]

Ventilators C, F, and G were the most reliable: bias was close to zero for these ventilators, with very narrow limits of agreement. For devices C and F, there was a statistically significant but clinically irrelevant relationship between bias and importance of leaks (by linear regression, a 60 L/min increase in leaks would increase bias by 3 and 5.3 L/min, respectively).

Conversely, devices A, B, D, and E underestimated the magnitude of leaks by an average of 6 to 26 L/min: for devices D and E, this could lead to a gross underappreciation of leaks. The results were intermediate for device B (a 60-L/min increase in leaks would induce a 12-L/min increase in bias). Bias was more important for devices A, D, and E, with wider limits of agreement and a clear relationship between bias and importance of leaks (for a 60-L/min increase in leaks, bias would increase by 21.7, 14, and 37.7 L/min, respectively). For device E, results were

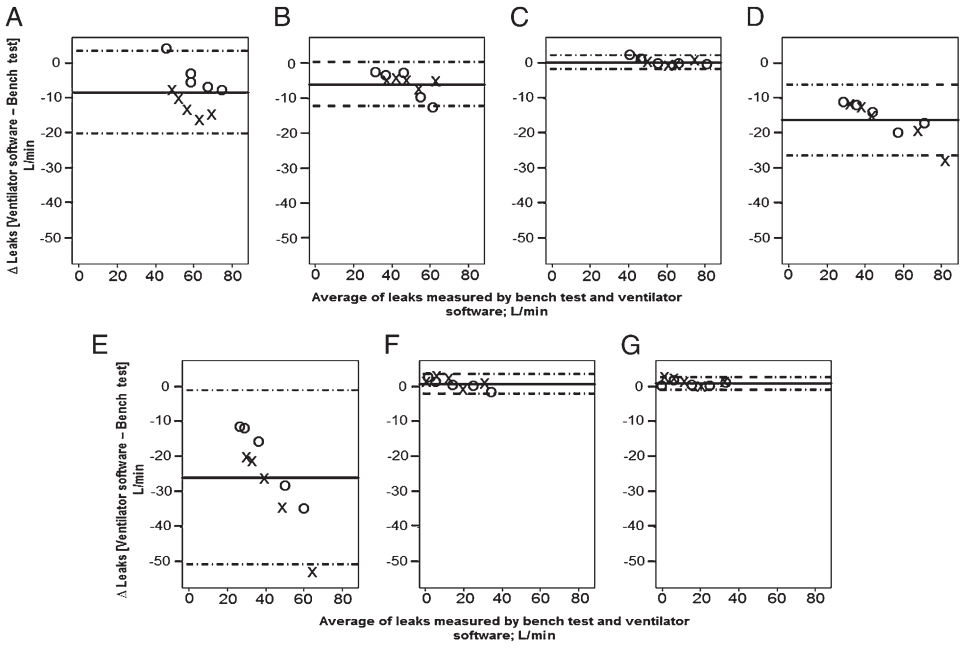

FIGURE 2. Chart shows data on leaks for the ventilators tested. *Y* axis describes [leaks from ventilator software − leaks from bench test data] in liters per min. *X* axis indicates average of leaks assessed by ventilator software and bench test, in liters per min, according to Bland and Altman.[20] ○ represent the values obtained at an inspiratory positive airway pressure (IPAP) of 15 cm H_2O. X represent the values obtained at an IPAP of 25 cm H_2O. Solid lines represent bias; dotted lines show limits of agreement (bias ± 1.96 × SD). For codes of ventilators (A-G), see Tables 1 and 2. Values obtained for devices F and G are without intentional (mask) leaks (see text for explanation). Manufacturers define (arbitrarily) clinically relevant leaks as > 24 L/min for devices F and G.

clearly unreliable: this device estimates leaks only during the expiratory phase.

Increasing leaks generated ventilator autotriggering in two devices (device D, with a maximal RR recorded at 25 cycles per min, and device E, with a maximal RR recorded at 19 cycles per min).

Tidal Volume

Figure 3 and Table 3 summarizes results for VT. VT was slightly underestimated by all devices (Table 3): the average bias for VT, all conditions considered, ranged between 66 and 236 mL; furthermore, limits of agreement (ie, difference between the fifth and 95th percentile of bias) ranged from 118 mL to 490 mL. Only two devices (B and C) had a bias < 100 mL.

For all devices, discrepancies in the estimation of VT increased significantly with IPAP (15 vs 25 cm H_2O). This difference was most important with device D, and importantly increased with increasing leaks.

Conversely, the relationship between ΔVT and leaks was not significant for three devices (A, C, and D), positive in one device only (E), and inverse in three devices (B, F, and G). Using multivariate linear regression for a given IPAP level and a 60-L/min increase in

Table 2—*Evaluation of Leaks*

Device[a]	Ventilator	Average Leaks (SD), L/min	Bias (SD), L/min[b]	Upper and Lower Limits of Agreement, L/min	R^{2c}	*P* Value
A	Monnal T30	63.8 (10.9)	−8.3 (6.1)	[−20.3; 3.7]	0.701	.003
B	Synchrony	50.5 (11.9)	−6.0 (3.2)	[−12.3; 0.3]	0.957	< .001
C	Trilogy	58.5 (13.6)	0.3 (1.0)	[−1.7; 2.3]	0.997	< .001
D	Ventimotion	58.2 (20.8)	−16.3 (5.2)	[−26.5; −6.1]	0.987	< .001
E	Vivo 40	54.8 (10.5)	−25.9 (12.7)	[−50.8; −1.0]	0.829	< .001
F[d]	VPAP III ST	14.6 (12.7)	0.8 (1.5)	[− 2.1; 3.7]	0.993	< .001
G[d]	VPAP IV ST-A	14.8 (12.5)	0.9 (0.9)	[−0.9; 2.7]	0.995	< .001

[a]Devices A-G refer to labels in Figure 2 and Figure 3.

[b]Bias (ventilator software − date from bench test) and limits of agreement are calculated according to Bland and Altman.[20]

[c]R^2 indicates variance of leaks explained by ventilator software.

[d]For devices F and G, values are unintentional leaks only (software of these devices report only unintentional leaks).

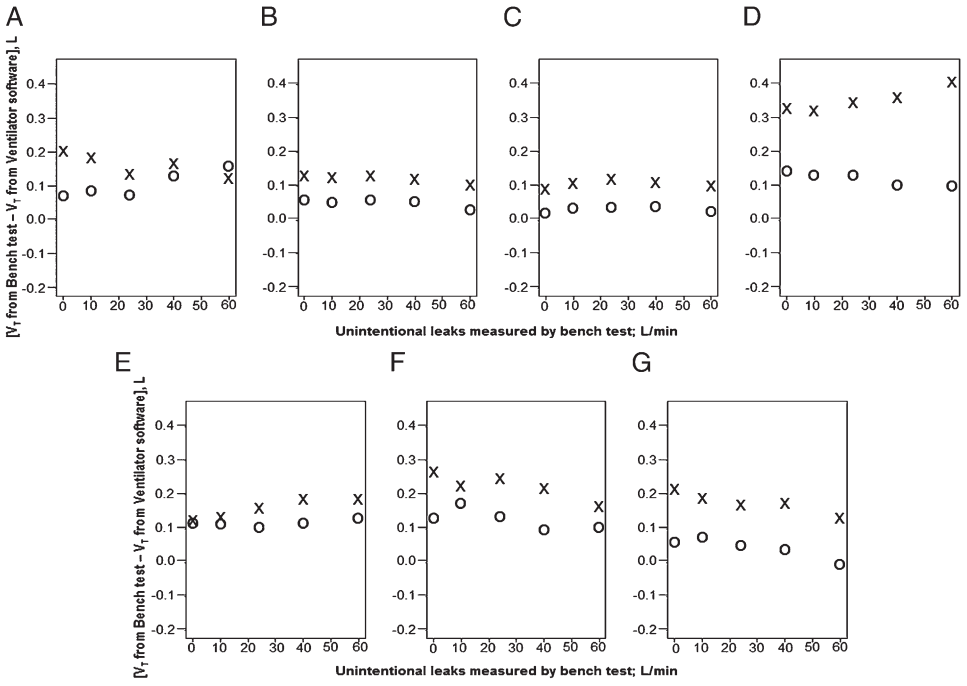

FIGURE 3. Estimation of V_T by ventilator software according to IPAP and leaks. *Y* axis describes [V_T from bench test − V_T from ventilator software] in liters. *X* axis indicates unintentional leaks assessed by bench test, in liters per min (from 0 to 60 L/min). ○ represent values obtained at an IPAP of 15 cm H_2O; X represent values obtained at an IPAP of 25 cm H_2O. See Table 3 for bias and limits of agreement and for codes of ventilators (A-G). V_T = tidal volume. See Figure 2 legend for expansion of other abbreviation.

leaks, bias for VT would be expected to increase by 12 to 126 mL, depending on the device tested.

Data obtained from ventilator software and bench test measurements for leaks and VT at the highest pressure-support value (IPAP, 25 cm H_2O), without unintentional leaks and with an unintentional leak of 60 L/min are shown in Table 4.

Discussion

The present study is, to our knowledge, the first to assess the reliability of two important parameters, leaks and VT, provided by the software of recent home bilevel ventilators by comparing results with objective assessment on a bench test. Results show the following: (1) different devices do not estimate leaks in the same way, that among seven ventilators tested, results provided were calculated in three different ways; (2) the precision of estimation of leaks varied very significantly between devices, such that results were excellent for three devices tested and average or even poor for four devices tested; (3) for four ventilators tested, bias for the estimation of leaks clearly increased with the importance of unintentional leaks; and (4) on average, VT was underestimated,

Table 3—*Evaluation of VT*

Device[a]	Ventilator	Average VT (SD), mL[b]	Bias (SD), mL[c]	Upper and Lower Limits of Agreement, mL[c]	$R^{2[d]}$	*P* Value
A	Monnal T30	658 (172)	130 (46)	[40; 220]	0.909	.045
B	Synchrony	696 (228)	84 (39)	[8; 160]	0.946	< .001
C	Trilogy	706 (223)	66 (40)	[−12; 144]	0.994	< .001
D	Ventimotion	809 (258)	236 (125)	[−9; 481]	0.991	< .001
E	Vivo 40	624 (208)	134 (30)	[75; 193]	0.983	.001
F	VPAP III ST	789 (267)	174 (61)	[54; 294]	0.989	< .001
G	VPAP IV ST-A	822 (311)	106 (75)	[−41; 253]	0.988	< .001

VT = tidal volume.

[a]Devices A-G refer to labels in Figure 2 and Figure 3.

[b]Average VT indicates pooled data measured by bench test assessment during all measurements performed.

[c]Bias and limits of agreement calculated according to Bland and Altman.[20]

[d]R^2 indicates variance of VT explained by ventilator software.

Table 4—*Results Obtained From Bench Test and Test of Ventilator Software for Seven Ventilators*

Device	VT Bench, mL[a]	VT Software, mL[b]	[VT Bench] − [VT Software], mL	Leaks on Bench, L/min[c]	Leaks from Software, L/min[d]	[Leaks on Bench] − [Leaks from Software], L/min
			Unintentional leak, 0 L/min			
A	912	711	201	52.8	45.0	7.8
B	968	840	128	40.1	35.0	5.1
C	886	797	89	44.8	46.0	−1.2
D	1,033	705	328	38.1	26.2	11.9
E	809	690	119	40.5	20.2	20.3
F[e]	1,015	750	265	0.0	1.2	−1.2
G[e]	1,032	820	212	0.0	2.4	−2.4
			Unintentional leak, 60 L/min			
A	668	547	121	76.8	62.0	14.8
B	800	700	100	65.5	60.0	5.5
C	923	826	97	74.3	75.0	−0.7
D	1,116	712	404	96.2	68.2	28.0
E	763	580	183	91.3	38.2	53.1
F[e]	1,062	900	162	30.4	31.2	−0.8
G[e]	1,228	1,100	128	32.5	33.6	−1.1

Ventilators were tested for VT and leaks at 25 cm H_2O of IPAP in two conditions: without unintentional leaks and with an unintentional leak of 60 L/min (at IPAP level). IPAP = inspiratory positive airway pressure. See Table 3 legend for expansion of other abbreviation.

[a]VT bench indicates VT measured on bench test.

[b]VT software indicates VT estimated by ventilator software.

[c]Leaks on bench indicates leaks measured on bench test.

[d]Leaks from software indicates leaks estimated by ventilator software.

[e]Devices F and G subtract intentional leak (mask) from total value of leaks

with a wide range of limits of agreement; for most devices, bias increased with higher IPAP levels, but was little affected by the importance of leaks.

The estimation of unintentional leaks is of major importance when monitoring NIV. Indeed, leaks have been reported in ≤ 34% of patients under stable, long-term NIV[8] and have per se a deleterious impact on the quality of sleep.[21,23,24] Leaks increase the probability of poor detection or nondetection of inspiratory efforts and may lead to unrewarded inspiratory efforts or other forms of patient-ventilator asynchrony[25] and to recurrent and/or prolonged episodes of desaturation and hypoventilation.[6] Although home bilevel ventilators have a high capacity of leak compensation, increased flow related to leak compensation can also be a source of discomfort. In this study, we first realized that data provided by ventilator software were not measured in the same way in all devices; this is not always explicitly stated by the manufacturers. One device estimated leaks only under expiratory positive airway pressure (device E) (these results are thus misleading and cannot be compared with those of other devices); furthermore, some devices subtract from their estimation of leaks the intentional leaks expected for a given type of mask at a given pressure setting (devices F and G),[22] while others report the sum of intentional and unintentional leaks. Once this was taken into account, the reliability of leak assessment was shown to be highly variable from one device to another. Three of the devices tested (A, D, and E) appear unreliable for monitoring of leaks, and may lead the physician to underestimate the importance of leaks in routine monitoring. The level of pressure support (two levels tested) did not seem to affect the estimation of leaks within the range tested.

The other parameter assessed was VT. The ability of a home ventilator to maintain a stable VT is related not only to the pressurization capacities of the device,[26] but also to its assessment of VT, especially in the presence of leaks.[13] A recent study has shown that four out of six devices designed to maintain a preset VT tended to underestimate VT.[13] Also, all but one of these devices failed to maintain a preset VT in the presence of unintentional leaks.[13] VT is an important parameter for adjusting ventilator settings and optimizing nocturnal ventilation. In agreement with Fauroux et al,[13] our data showed that all ventilators tested tended to underestimate VT; furthermore, underestimation of VT increased significantly at higher pressure-support levels (two levels tested), and this difference was marked in one ventilator (device D). Bias for VT, all conditions combined, ranged from 66 to 236 mL, thus introducing, for some devices, a considerable possibility of error in adjusting ventilator settings. Because data provided by software tend to underestimate VT, this can lead physicians to increase pressure support, which in turn can aggravate leaks.

This study has a few limitations. First, we chose to analyze the reliability of only two items: leaks and VT.

Minute ventilation, when provided by ventilator software (as in five out of the seven ventilators tested) is estimated using RR and Vt. (RR was shown to be very reliable in all conditions in this study, but this should be confirmed over a wider range of frequencies.) Other items such as the apnea or apnea-hypopnea index, alarms, inspiratory time/expiratory time ratio, percentage of spontaneous inspiratory triggering, or expiratory cycling are only provided by some ventilator software.[27] Furthermore, leaks and Vt are clearly the most relevant of these parameters. Second, we chose to perform this study using two different pressure settings only. This reflects the range of pressures prescribed for most patients under long-term NIV.[1,28] Third, there are inherent limitations to bench testing per se: indeed, the model does not integrate the complex variations that occur in breathing, inspiratory effort, and respiratory drive, and the inherent variability of unintentional leaks in clinical situations.[12,29,30] Fixed leaks are quite different from variations that occur in real life related to changes in position or variability of upper airway resistance. Also, the resistance of the bench circuit was different from that of in vivo studies: the addition of a "Y" valve and a low sensor between the mask and ventilator and, more importantly, the tubing of the "trachea" may impact on the total resistance of the circuit.[31] Taking into account these limitations, there is a body of literature that suggests that bench testing is, however, an important and relevant component of the assessment of home ventilators. Finally, our findings are highly dependent on the existing individual designs of the devices when we tested them and the conditions simulated. One must also consider that adding oxygen to the ventilator circuit may potentially modify these results.

In conclusion, data provided by ventilator software can be a useful adjunct to information provided by pulse oximetry, nocturnal capnography, and arterial blood gases, and an important contribution to the monitoring of long-term domiciliary NIV.[3] However, the physician must be aware of the lack of standardization in the reporting of results (leaks) and the important variability in the reliability of results provided according to the device used, with both items being potentially misleading. Items that have, to our knowledge, not yet been independently assessed, such as apnea or apnea/hypopnea indices, must clearly be evaluated, both on the bench and clinically, by comparison with polysomnography. The relevance of other items provided, such as the percentage of cycles triggered or cycled by the patient, must also be clarified. Finally, a consensus between manufacturers on the way of measuring and reporting data would be helpful.

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Dr Combescure: contributed to the analysis of data, statistical analysis, and revision of the manuscript.
Dr Pepin: contributed to the study design and protocol, analysis of data, and writing and revision of the manuscript.
Dr Jolliet: contributed to the study design and protocol, analysis of data, and writing and revision of the manuscript.
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